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LETTER FROM THE EDITORS

Again this past year the Editors and members of the Editorial Board have called upon many of their colleagues and others especially qualified to review certain manuscripts submitted to the JOURNAL for publication. They are most grateful for the help given them by the individuals whose names are listed below.

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The Editors wish to take this opportunity to express their appreciation to Dr. George E. Burch, Dr. Jerome W. Conn, Dr. Douglas A. MacFadyen, and Dr. Irvine H. Page for their high quality of performance and diligent service as members of the Editorial Board for the past several years. They will be replaced on the Board as of Jan. 1, 1952, by Dr. S. Howard Armstrong, Jr., Dr. A. C. Corcoran, Dr. Eugene Ferris, and Dr. Randall G. Sprague, who have accepted three-year appointments.

The Editors gratefully acknowledge the excellent service and cooperation of the Mosby Company during the past three years. They would also like to commend Mrs. Lois Richards of the University of Chicago for the superior way she handles the hundreds of manuscripts and thousand of letters in the Editor's office.

The work of reviewing and publishing manuscripts can oftentimes be made easier if the authors would pay more attention to organization of the data. Manuscripts organized under the following general headings are most easily evaluated: Introduction, Materials and Methods, Results, Discussion, and Summary.

This JOURNAL requires the academic degrees of the authors and complete bibliographic references in manuscripts. When these are incomplete, which is not infrequent, there is, of necessity, a delay in processing the manuscript for publication. For detailed information concerning the preparation of manuscripts the authors are referred to the Editorial Communications page usually found on the inside of the back cover of the JOURNAL.

—*Clayton G. Loosli, M.D.*
Editor
—*Matthew Block, M.D.*
Associate Editor

A TESTAMENT OF DUTY

Some Strictures on Moral Responsibilities in Clinical Research

William Bennett Bean, M.D.
Iowa City, Iowa

IT IS the immemorial custom of societies such as ours to hold an annual clambake; and the president is expected to invoke his prerogative of perpetrating an address. Some have enlivened the occasion with clownish antics, some with solemn beard tugging, or scowling with alarm at things as they are. Others have taken broad and panoramic views over particular aspects of the inexhaustibly various fields of clinical research. Frustrated investigators have even used the opportunity to give a paper which former incumbents of the office had omitted from previous programs.

I propose to depart a little from established patterns and discuss with you a simple problem at the very heart of clinical research, namely, the moral responsibilities of the clinical investigator. Though the Cassandras of moral decay are now in vogue, I was pondering this topic well before the current fashion yielded such a ripe harvest. As far as I can find, morality has not been the theme of a presidential talk before this or any other society concerned with clinical research. Perhaps others have not thought it appropriate but rather extraneous to the objective investigator, or have shied away because of indifference or pride masquerading as humility. I cherish this office as a great honor and opportunity, and will try to live up to it dealing with problems of deep significance for us physicians, investigators, and teachers. While calling attention to a situation will be my chief concern, some suggestions for possible treatment and salvage will be offered. The diagnosis clearly seen anticipates the cure; or at least defines the prognosis for humble contemplation. It would be disingenuous to imply, in setting forth certain moral obligations as essential in the creed of physicians of good will, that I have succeeded always in following my own precepts.

MORAL RESPONSIBILITY IN HUMAN EXPERIMENTS

Experiments on man are perhaps coterminous with human existence, in wars and accidents, therapy and research. In ancient Persia, kings gave over condemned criminals for experiments useful to science. Later this was practiced by the Ptolemies in Egypt, and during the Renaissance by Fallopius in Pisa. Let me introduce some thoughts of Claude Bernard on this subject.

"As far as direct applicability to medical practice is concerned, it is quite certain that experiments made on man are always the most conclusive. No one has ever denied it. . . . First, have we a right to perform experiments and vivisections on man? Physicians make therapeutic experiments daily on their

From the Department of Internal Medicine, State University of Iowa.
Presidential address, Twenty-fourth Annual Meeting of the Central Society for Clinical Research, Chicago, Ill., Nov. 2 and 3, 1951.

patients, and surgeons perform vivisections daily on their subjects. Experiments, then, may be performed on man, but within what limits? It is our duty and our right to perform an experiment on man whenever it can save his life, cure him, or gain him some personal benefit. The principle of medical and surgical morality, therefore, consists in never performing on man an experiment which might be harmful to him to any extent, even though the result might be highly advantageous to science, i.e., to the health of others. But performing experiments and operations exclusively from the point of view of the patient's own advantage does not prevent their turning out profitably to science."

Clinical investigators rarely meditate upon the wide cleavage which separates clinician from investigator in their split personality. As physicians, their prime concern is intimate, personal responsibility in caring for sick people. As investigators they are goaded by divine discontent and impelled by curiosity as well as ambition for renown. Such stimulus sometimes suppresses the physician altogether. The different views are seen well in the subtle but great change which occurs when a house officer becomes a research fellow. Unless wisdom and compassion are neatly blended, the friction between the excited investigator bent on research and the resident with his mind set on care for the patient may produce fire as well as acrid smoke.

One of the causes for a too casual view of the patient as subject is the education of clinical investigators, their background, and their purposes. In times gone by clinical research was carried on by physicians whose orientation was general practice, the clinic or hospital. Their training was more in the art of practice than the science of the laboratory. Problems arising in the context of sick bed or office, exemplified by clinical disease in a puzzling patient, provided incentive to seek a solution by whatever means were at hand. Clinical research, facing the problem of puerperal sepsis, gave a solution by Oliver Wendell Holmes which epitomized the utility of careful empiricism before Koch and Pasteur provided exact science which put the mechanisms on a higher level of comprehension. In no sense did science change the implications. Long before this, Lind had found how to prevent scurvy. The facts, dangling before the averted eyes of chemists and physiologists, had to wait a century and a half for detailed explanation. Walter Reed discovered the manner of yellow fever's epidemic spread before the laboratory had defined its specific filterable virus. In more recent times the clinical response of pernicious anemia to liver was clear fact well before the missing factor was isolated. In the contemporary field clinicians provided insight into the functions of cortisone and ACTH, while it was puzzling those whose imagination was hemmed in by the traditional philosophy of the laboratory.

Aside from the time lost in the desultory and idle ritual of experiment devoid of ideas, what further implications are there in the orientation of experimental medicine away from the sick person into the labyrinths of the laboratory? First is the artificial cause of research where technical vertuosity, giving method, directs the energies of investigators into predetermined channels whose boundaries are hedged about by a technique. Applied research

is of great use. It does not open new lines of thought. Unless fertilized by new ideas, it imprints a rigid pattern but never alters the course of practice by bold novelty or originality. In the end it is stultifying to its practitioners and becomes formalized in the empty echoes of a sequence of pedestrian projects.

Of more danger and concern is the custom now current that clinical investigators come to positions of academic responsibility by apprenticeship in laboratory science rather than by the thornier path as practitioners of the art who become wise clinicians. And the danger is not in the matter of teaching, which may be done well or poorly without exact correlation with training, but that the most praiseworthy zeal for knowledge may lead the man whose technical background overshadows his caring for the patient into a disregard for the subject of his researches. Thus, potentially dangerous experiments may be done without the subjects' knowledge or express permission. Whether it be thoughtlessness or heartlessness, such practice is a measure of the moral obliquity which exists in some high places of research today. Fortunately, it is not widely current, but it does exist. At a time when ethical standards are high, or religion elevates moral tone, this situation would have other correctives. They are not effective today. Moral necrosis is sinister in its pervasive insinuations, and all who are concerned with clinical research, that is, experiments on themselves and their fellow man, must face the implications. The recent degradation of physicians in Nazi Germany exemplifies the decline and fall of a group whose moral obligations went by default in a single generation. The house would not have fallen had not many timbers been rotten. Descent into the gas chamber by doctors of infamy had its beginning in disregard for the patient. Never forget that the difference between an experimen on human beings without clear understanding and freely granted permission, and the determination of the M.L.D. in man is one of degree, not of kind. The patient, however humble and however ill, in whatever degree derelict and forlorn, has sacred rights which the physician must always put ahead of his burning curiosity.

THE MORAL RESPONSIBILITY TO HELP THE RISING GENERATION OF PHYSICIANS

Clinical investigators have a deep obligation as teachers to encourage younger associates and students to follow a career and achieve excellence worthy of the best of the profession of medicine. I was disheartened recently when a leading clinical society effectually excluded from attendance the intern-resident group, the very ones most likely to gain inspiration and benefit from their proceedings. Though this mournful situation was eagerly corrected when called to their notice, the fact that it was permitted to happen suggests a serious lack of vision. It indicates the real need for championing our juniors if we are to raise up admirable followers. (That this could fail by default may be read as an omen of the degree to which minutiae or trivia occupy the councils of those to whom we have looked for leadership.)

A stifling influence of still graver import, one that no clinical teacher has willingly tilted at in public for reasons all too apparent, is the overweening and crushing impact of the specialty boards. As a theme recall what Lord

Acton said—"power corrupts and absolute power corrupts absolutely" and Burckhardt's admonition "power is of its nature evil, whoever wields it." Also let it be recognized in charity that my comments are directed at that anonymous corporate being, the Board of Internal Medicine, not individual members whom we would all cheerfully confess to be our leaders in clinical medicine today. This corporate entity has a number of sinister aspects, and some of its acts can be described only as immoral. First, it is a self-appointed, self-perpetuating body without status in tradition or law, but with imense prestige in practice.* If it is representative of American medicine, or even its better aspects, this is only accidental. It sits as a judicial, executive, and legislative body, but with no semblance of a democratic basis, no external critique, no constant rejuvenation from youthful sources. Thus its very composition incrusts it with conservatism and focuses its procedures on a backward view of medicine or at best the status quo. But unless medicine is constantly advancing it retrogrades. Harboring in the quiet estuaries of the past is a poor index of capacity to go forward. It is a sheet anchor against advance out on the high seas. There is the tacit assumption that regimented, arbitrarily set training guarantees excellence in practice and maturity of judgment, an assumption we see contravened every day. If anything, such training defeats its own supposed ends.

Even if one condoned the general purpose of the Board, how far does it fall short of achieving its goal? What incentive is there to further advance or even keep up, after the hurdle is crossed? Once a board man always a board man. Permanent status rewards temporary achievement and this fault is common to state licensing bodies and other boards, rendering their pompous concern with standards eminently silly. There is no recurring measure of progress, no "quinquennial brain dusting," no lustrum to refresh and purify. The encouragement is to run one race and stop.

With this price tag on passing, how does the Board reach its ineluctable and ineludible decisions? Are the criteria for admission as valid today as the academic benchmark used for those who were arbitrarily proclaimed satisfactory specialists at its commencement? No effort is made to find out what the candidate really understands, but only what fraction he knows of what the examiner knows. Because of the mass of would-be specialists, one set of examinations is of the multiple choice variety, as though medical learning could be reduced to a clear and final choice among a few rigid alternatives. The data are easy to get, but what wise man understands what they mean? And here a fearful phenomenon comes into play. At least on some occasions no absolute criterion of satisfactory performance determined passing or failing, but a locus on a distribution curve made up of the scores of a particular group of candidates. The same absolute performance which passed one time failed another. Success was determined not by one's own efforts, but by what others

*It has been pointed out to me that appointments to the Board are made by the American College of Physicians and the Section of Internal Medicine of the American Medical Association. Thus, manifestly it is not "self-appointed and self-perpetuating" and the onus should be placed where it belongs. I sincerely regret my error. Nevertheless, this body must be held responsible for its deeds and its philosophy and their implications. It seems best to leave the remarks as delivered without change.

did. We can hardly find a worse example of moral irresponsibility than standards built upon such a morass. What possible expediency can justify herd performance as criterion of excellence? Is it not a curious and depressing thing that in America, where the look has been to the future, the incorrigibly powerful Boards should combine the weakest and retrospective aspects of French faith in scholasticism, British faith in examinations, and Teutonic faith in degrees?

Unfortunately, the Boards have spawned a strange parasite, the ambitious but immature specialist, more interested in the tinsel and trappings of his career than in his true work of becoming the best possible physician. Originality is not encouraged. Even if the aims were equitably reached, the discovery of competence is a far cry from the prophecy of excellence.

An unrealistic feature of the system is the practical fact that those who have invested the years of training required for admission to the examination will practice as specialists whether they pass or not.

A by-product of this system of reactionary stereotypy, built on profound faith in examinations, those hallmarks of academic omphalism, is the dictation of educational terms to medical schools and hospitals already burdened with financial problems they cannot meet. This is done without any overt practice of inspecting to given assurance that standards are kept up, or reviewing to judge the quality of the programs required, or implementing dictates with a budget.

In fine, the Boards have usurped the rights and function of our universities. They sit harpie-like with Molotovian veto on progress, perhaps elevating the mass but stifling activity at the shifting interface between knowledge and ignorance where imagination probes with its innumerable patrols into the unknown. In my opinion it is high time that the universities got back their ancient prerogatives of graduate training, and that medical education be in the hands of teachers, not specialists. Only thus can we hope that originality will lead its cherished few to excellence, and youth will redeem the elders by advancing progress.

THE MORAL OBLIGATION OF TEACHING

By implication if not by definition a clinical investigator must be a teacher. By a curious distortion of values capacity for teaching is more likely to be a handicap than a help to a young person who aspires to be a good clinical teacher. Perhaps this paradox results from failure to take seriously the main responsibility of a clinical chief, which is teaching. As medical teachers are chosen today with the eye asquint upon volume (and not necessarily quality) of published papers, it is accidental if superior talent for teaching adorns the odd assortment of characteristics demanded of medical department heads. No one has dared confront the curriculum head on and align it with the vast and incomprehensible array of new facts not yet codified in any sensible order. Always it is add another appendage. Unless a revival of integration permits wholesale pruning and sensible grafting the academic tree, overburdened with miscellaneous fruit, will fall of its own weight and spoil the harvest.

What is the most effective general teaching today at the postgraduate level? In sorrow we must admit that the artistic and artful brochures of wealthy pharmaceutical houses, sped on by a crusading band of detail men, have effectively taken over graduate teaching. The blandishments of advertising, siren song of the purveyor of pills, now that there really is a multitude of specifics, puts professional judgment in a sorry place. Harnessed to the lightning strokes of lay publicity, the demand for new miracle drugs often comes from radio or newspaper coaching, and the practitioner, fearing to "be not the first by whom the new is tried" becomes party to a conspiracy of ignorance, fraud, and twisted idealism which has run the gamut from vitamin craze to spurious cold cures and Hadacol. After all, we have some responsibility in this mess, and must provide leadership to protect the public and embellish the name of medicine. Certainly many pharmaceutical houses are advancing the cause of medicine. More power to them. I do not grudge the honest dollar to the shareholders in drug enterprises, but when their advertising budgets exceed the total outlay for teaching and research provided by all our medical schools concern is justified, "for where your treasure is there will your heart be also."

THE MORAL RESPONSIBILITY TO BE INTELLIGIBLE

Clinical research is predicated upon the belief that its significant results should be communicated and used by others. How miserably this is accomplished is any contemporary editor's tale of woe and any thoughtful reader's sorrow. The pseudo prestige of long and difficult words transcends the useful scientific term and diffuses widely through our papers. Simple things are made complicated, and the complex is made incomprehensible. Chaos reigns. The so-called medical literature is stuffed to bursting with junk, written in a hopscotch style characterized by a Brownian movement of uncontrolled parts of speech which seethe in restless unintelligibility. Every day we realize that the iron curtain which disbars us from sampling in adjacent fields of science is not so much the erudition of our colleagues as the tropical jungles of verbiage and gobbledegook in which this erudition lurks, unobserved save by the initiated. Has this unfortunate situation any corrective? If some small fraction of the time and effort which goes into the techniques of research were spent on study and perfection of the simple techniques of writing and speaking clearly, paths could be made in the jungle. Those who start late must read and study good models of exposition. Learn the simple rules: write, rewrite, delete, polish. For sage advice, Allbutt's "Notes on the Composition of Scientific Papers" has lost none of its cogency, and elegantly combines precept with example. For a contemporary view Gower's "Plain Words" is equally good. With such guides our scientific writing must improve. Correct grammar, thoughtfully combined with rhetoric, might lead through grace to that elusive quality style and make a worthy medium for telling of significant work.

THE MORAL RESPONSIBILITY FOR HIGH STANDARDS

Clinical investigators have a moral obligation to set standards of excellence, for who is to provide the profession with direction and tone unless

it be clinical investigators and teachers? Lest I try your patience with further diatribes sounding my philippic against moral laxity, I will simply list a few more present-day problems. If you ponder them and are content with what you find, my message falls on the stony ground of callousness or in the fetid swamp of indifference. First there is the responsibility to be *critical*, for without objective and wise criticism we are all misled by first looks and superficial appearances. And we must impart such a philosophy to our students. We must be critical on the subject of the *normal* with its multiple and sometimes mystic meanings, the *control*, elusive figment on whose shifting quicksands we try to erect our scientific temples of reason, and *multiple causes* a complex and relentless goddess we have forsaken in a riot of oversimplification in search of a defunct monism—the simple efficient cause. Lack of precision in techniques of communication aggravates the difficulty and gets scant antidote in measuring the readily measured, with ease of measuring the only and inconsequential incentive. *Statistics* when used to conceal poor planning or to warp data into preconceived patterns may be a seductive danger rather than a ready help. We must manage our manipulations of data or be led about willy-nilly by the nose. We must know the *medical history of disease*. Santyana said that those who are ignorant of history are doomed to repeat it. What needless waste of time might have been saved had the starry-eyed and naive repeater of old experiment done enough searching to know his subject. We must know the *natural history of health and of disease*, for how are we to separate the changing facets of existence unless we have clear eyes and cool judgment to discern ill from well? How many times do we see false cures praised when natural responses deserve the only credit, spurious claims which would be avoided if the observer had knowledge born of experience? In the nature of things the beginning investigator gains something by youth, inexperience, and even ignorance of all that vast sea of errors which marks the slow and painfully charted course of progress. The senior investigator, however, must be the helmsman who keeps speed moderated and a sharp lookout while the new charting goes on.

Now I recapitulate and have done. Morality is keystone in the arch of medicine, supported by and joining the pillar of art and the pillar of science. In clinical research we must do experiments on our fellow man, but they are justifiable only with freely granted permission, and we must forego them when they conflict with the interests and rights of our patients. We must foster the rising generation of our successors, and return the rights and duties of medical education to the universities, diminishing the power of boards and the seduction of advertising as determinants of graduate training. We must fulfill our obligations as teachers as well as investigators. No longer can we shun our responsibility to be intelligible. And the duty of each of us includes the need to sustain high standards of excellence by exercising with what courage we may the unpleasant and hard task of criticism. Only when humanity regulates research, when originality is not constricted by arid conformity, when duty clearly seen is cleanly met, can we hope to live up to the simple but wonderful purpose of our society—"The cultivation of clinical research and the development of the younger men."

BLOOD PRESSURE IN THE AORTA DURING RESECTION, AND REPAIR OF COARCTATION OF THE AORTA*

JOSIAH FULLER, M.D.,** BOWEN E. TAYLOR, M.D.,***
O. THERON CLAGETT, M.D.,§ AND
EARL H. WOOD, M.D., PH.D.||
ROCHESTER, MINN.

SINCE surgical resection and repair of coarctation of the aorta, an operation involving considerable technical risk, has been done for only a relatively few years, it is impossible to be certain how the procedure will affect the prognosis of the disease. For this reason, numerous physiologic tests have been performed on patients preoperatively and postoperatively. Brown and co-workers[3] and Bing and his colleagues[2] have reported studies of intra-arterial pressure in the arm and leg before and after operation. Since the studies by Brown and co-workers[3] did not always show the expected lowering of blood pressure, particularly in the subclavian type of anastomosis, we decided to study the immediate effects produced during the operation by a more or less complete removal of the aortic stricture. It was thought that a study of the pressure changes and circulatory dynamics in the aorta associated with this sudden change might shed some light on the disturbed pressures and circulatory dynamics associated with this condition.

MATERIALS AND METHODS

The mobile oscillographic camera¶ developed by Hallenbeck and associates[9] was used to record multiple physiologic variables during nine operations for surgical repair of coarctation of the aorta in man. Simultaneous recordings were obtained of the electrocardiogram, respiration, heart rate, and direct blood pressure in the radial artery. By use of the technic described by Taylor and his colleagues,[17] it was possible to record the blood pressure directly through a 25-gauge needle introduced into the aorta above and then below the stricture before and after resection and end-to-end anastomosis of the aorta.

The strain-gauge† manometer systems[13, 15] used were damped both mechanically by using small-bore needles and by electromagnetic damping of the Heiland type A galvanometer‡ after the manner described by Lambert and co-workers.[13-15] The dynamic responses of the complete strain-gauge manometer systems used were determined by means of a pressure oscillator[12, 14, 20] of variable frequency and by recording the responses resulting from a square-wave change in pressure in the manner described by Lambert and Jones.[14] Fig. 1 illustrates the step response produced by subjecting the manometric system used for recording intra-aortic pressure to a square-wave pressure change. Fig. 2

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§Division of Surgery.

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TABLE I. AVERAGE OF DYNAMIC RESPONSE CHARACTERISTICS OF MANOMETRIC SYSTEMS USED FOR RECORDING INTRA-AORTIC AND INTRARADIAL ARTERIAL PRESSURES

MANOMETRIC SYSTEM	RESPONSE TO SINE-WAVE PRESSURE CHANGES OF VARIABLE FREQUENCY: STATIC DEFLECTION AT DESIGNATED FREQUENCIES IN CYCLES PER SECOND (PER CENT)					RESPONSE TO SQUARE-WAVE PRESSURE CHANGE		
	10	15	20	25	30	RESONANT FREQUENCY (CYCLES PER SECOND)	DEFLECTION TIME (SECONDS)	DAMPING[4, 11, 22] COEFFICIENT
Aortic	106	107	108	103	95	29	0.02	0.62
Radial	105	106	109	109	102	35	0.02	0.61

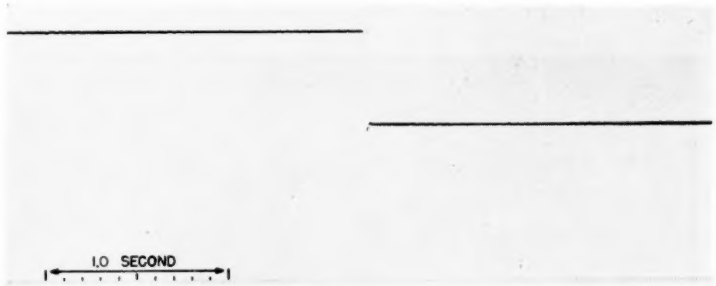

Fig. 1.—Step response (square wave) of manometric system similar to that used for recording pressures in the aorta. The Statham strain-gauge manometer model P6-15D-250 was connected directly to a curved 24-gauge needle 45 mm. long. A Heiland type A galvanometer was used with a total external damping resistance of 108 ohms. Calculated from the figure: deflection time, 0.02 second; resonant frequency, 30 cycles per second; damping coefficient, 0.66.[4, 11]

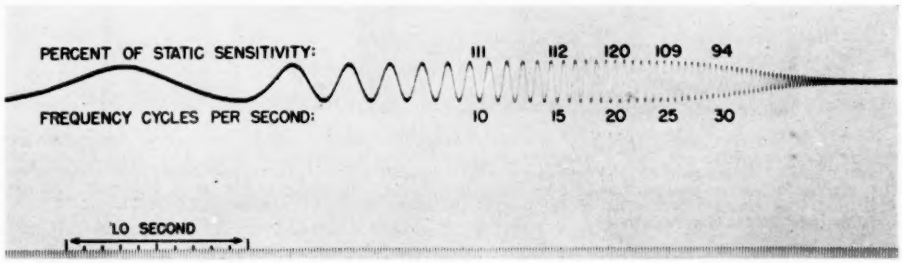

Fig. 2.—Variation in response of manometric system to equal-amplitude sine-wave pressure variations of increasing frequency. Pressure variations were produced by a mechanical hydraulic oscillator.[12, 22] The manometric system is described in the legend for Fig. 1.

portrays the response of this manometer system when subjected to sine-wave pressure changes of variable frequency produced by the pressure oscillator. Table I gives the average of the dynamic responses obtained from the manometric systems used for recording the pressures in the aorta and in the radial artery. The dynamic sensitivities of the aortic and radial manometric systems were within ± 9 per cent of static sensitivity up to 30 cycles per second with diminishing sensitivity thereafter. The time delay of these manometer systems to an applied square-wave pressure variation was 0.005 second. The time delay of the electrocardiographic recording assembly was not measurable at the chart speed of 25 mm. per second used in these studies. It is believed that manometer systems with these characteristics are adequate for reasonably accurate recording of arterial pressure pulses.[5, 20, 21]

Blood pressures were measured from the records according to the usual technique for physiologic studies of blood pressure. The systolic pressures were taken as the highest point on the pulse pressure curve and the diastolic pressures as the lowest point just preceding the systolic upswing. The mean pressures were calculated by planimetric integration. For any of the reported blood pressure values, the continuous pressure recordings were measured for at least a five-second period and the average pressures for the period used.

The average age of the nine patients considered in this study was 26 years, with a range of 11 to 38 years (Table II). The known duration of hypertension averaged six and one-half years, but varied from five months to seventeen years. No evidence of renal abnormality was noted in any of these cases. Funduscopic examination revealed some arteriolar narrowing in all the cases in which the examination was made. There was evidence of minimal sclerosis in only one case.

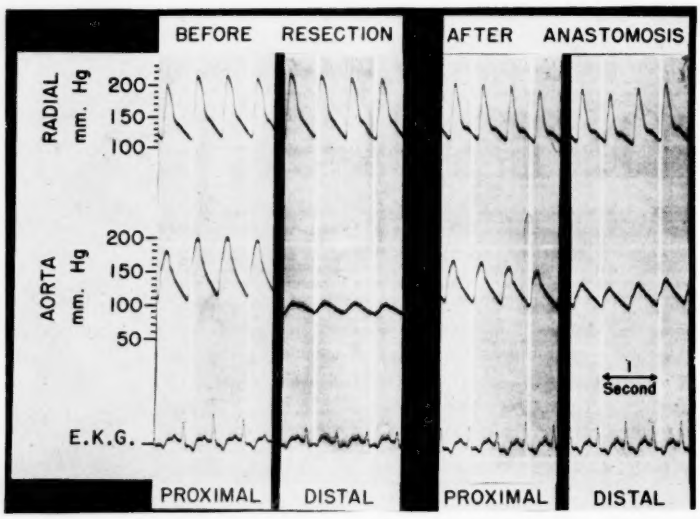

Fig. 3.—Direct blood pressure in the right radial artery and in the aorta during surgical repair of coarctation of the aorta. The radial arterial pressure was 208 mm. of mercury systolic and 115 diastolic before resection, and 193 and 113 after completion of the anastomosis. The proximal aortic pressure was 204 systolic and 120 diastolic before and 173 and 113 after repair of the coarctation. The distal aortic pressure was 108 systolic and 93 diastolic before and 135 and 102 after repair.

TABLE II. DATA ON 9 PATIENTS ON WHOM BLOOD PRESSURE DETERMINATIONS WERE MADE DURING OPERATION FOR COARCTATION OF THE AORTA*

PATIENT	AGE (YEARS)	KNOWN DURATION OF HYPERTENSION (YEARS)	FUNDUSCOPIC EXAMINATION
1	18	5 mo.	Slight arteriolar narrowing
2	20	1	Moderate arteriolar narrowing
3	24	6	Moderate arteriolar narrowing
4	38	17	Moderate arteriolar narrowing
5	25	4	Not done
6	27	7	Minimal arteriolar narrowing
7	34	8	Slight arteriolar narrowing
8	11	Not known	Not done
9	33	15	Moderate arteriolar narrowing; minimal arteriolar sclerosis
Average	26	6½	
Range	11-38	5 mo.-17 yr.	

*Evidence of renal abnormality was absent in all cases.

RESULTS

Simultaneous recordings of the electrocardiogram and of arterial pressure in the arch of the aorta and the radial artery (Tables III and IV, Fig. 3) indicate that the onset of the aotic pressure pulse proximal to the coarctation before repair, occurred 0.10 (range 0.08 to 0.12) second after the onset of the R wave of the electrocardiogram. This time interval includes the greater instrumental time delay (0.005 second) of the aortic manometer assembly. Therefore, the actual time interval from onset of the R wave to the onset of the pressure increase in the aorta would be approximately 95 milliseconds. The onset of the right radial pulse, before repair of the coarctation, occurred 0.07 (range 0.05 to 0.09) second after the onset of the pulse in the proximal aorta. The average systolic pressure in the arch of the aorta during repair but before resection was 156 (range 125 to 204) mm. of mercury. However, for the same heartbeat the systolic pressure in the radial artery exceeded that in the proximal aorta on the average by 14 (range 2 to 40) mm. of mercury. Likewise, the radial pulse pressure exceeded that in the proximal aorta by 20 (range 6 to 43) mm. of mercury. As would be expected, the diastolic and mean pressures in the radial artery averaged less than the pressure in the aorta. The differences were 6 (range 3 to 10) and 7 (range 5 to 10) mm. of mercury, respectively.

Simultaneous recordings of the pressure in the radial artery and in the aorta just distal to the site of the coarctation, made during repair but before resection (Tables III and IV, Fig. 3), indicate that in seven cases the onset of the radial pulse actually preceded the onset of the pulse in this section of the aorta by 0.02 (range 0.01 to 0.04) second. The systolic, diastolic, pulse and mean pressures in the radial artery exceeded the corresponding pressures for the same heartbeats in the distal aorta on the average by 76 ± 9*, 12 ± 2, 66 ± 8, and 31 ± 4 mm. of mercury, respectively.

A comparison of the ratios of the pressures in the proximal aorta and in the distal aorta to the pressure in the radial artery was made during repair of the coarctation but before resection (Table V). The ratio of the proximal aortic to the radial systolic pressure was 0.92 ± 0.02 as compared to 0.55 ± 0.06 for the distal aorta. The ratio for mean pressures was 1.06 ± 0.02 for the proximal aorta and 0.74 ± 0.06 for the distal aorta. The pulse pressure ratios showed the greatest differences, the average ratio of the proximal aortic to the radial pulse pressure being 0.75 ± 0.13 as compared to 0.12 ± 0.03 for the aorta just distal to the site of coarctation.

Occlusion of the aorta at the site of the coarctation in four cases (Figs. 4 and 5) was without apparent effect on the radial arterial pressure or on the aortic pressure above or below the stricture site. It is concluded that the volume of blood flowing through the coarctation was not sufficient to contribute significantly to the maintenance of blood pressure in the distal aorta.

Occlusion of the left subclavian artery in the chest in four cases (Figs. 4 and 5), however, produced an immediate increase of the systolic and pulse pressures in the radial artery and proximal aorta averaging 17 mm. of mercury

*The numbers following the ± signs are the standard errors of the mean.

TABLE III. EFFECT OF REPAIR OF COARCTATION OF THE AORTA ON THE CONDUCTION TIME OF THE PULSE WAVE IN THE AORTA AND RADIAL ARTERY

	AORTA PROXIMAL TO COARCTATION						AORTA DISTAL TO COARCTATION					
	SECONDS FROM R WAVE* TO ONSET OF AORTIC PULSE			SECONDS FROM ONSET OF AORTIC PULSE TO ONSET OF RADIAL PULSE			SECONDS FROM ONSET OF AORTIC PULSE TO ONSET OF RADIAL PULSE			BUILD-UP TIME OF AORTIC PULSE WAVE, SECONDS		
PATIENT	BEFORE REPAIR	AFTER REPAIR	DIFFERENCE	BEFORE REPAIR	AFTER REPAIR	DIFFERENCE	BEFORE REPAIR	AFTER REPAIR	DIFFERENCE	BEFORE REPAIR	AFTER REPAIR	DIFFERENCE
1	0.10			0.09			−0.03			0.14		
2		0.07			0.10							
3	0.08	0.07	−0.01	0.06	0.06	0	−0.02	0.06	0.08	0.18	0.16	−0.02
4	0.10	0.11	0.01	0.08	0.08	0	−0.01	0.06	0.07	0.22	0.11	−0.11
5		0.11			0.05		−0.01	0.04	0.05	0.23	0.19	−0.04
6	0.12	0.11	−0.01	0.05	0.07	0.02	−0.03	0.04	0.07	0.21	0.12	−0.09
7		0.11			0.09		−0.04	0.07	0.11	0.16	0.15	−0.01
8	0.09	0.10	0.01	0.05	0.06	0.01	−0.03	0.05	0.08	0.14	0.16	0.02
9	0.11	0.11	0	0.07	0.07	0		0.06			0.12	
Average	0.10	0.10	0	0.07	0.07	0.01	−0.02	0.05	0.08	0.18	0.14	−0.04

*R wave of electrocardiogram.

TABLE IV. COMPARISON OF AORTIC PRESSURE ABOVE AND BELOW THE STRICTURE WITH RADIAL ARTERIAL PRESSURE DURING REPAIR OF COARCTATION OF THE AORTA BEFORE RESECTION

	PRESSURE, MM. OF MERCURY											
	SYSTOLIC			DIASTOLIC			PULSE			MEAN		
PATIENT	1 RADIAL*	2 PROXIMAL AORTA	3 DISTAL AORTA	1 RADIAL	2 PROXIMAL AORTA	3 DISTAL AORTA	1 RADIAL	2 PROXIMAL AORTA	3 DISTAL AORTA	1 RADIAL	2 PROXI-MAL AORTA	3 DISTAL AORTA
1	187	165	97	98	107	87	89	58	10	130	137	94
3	208	204	108	115	120	93	93	84	15	148	156	101
4	136	130	72	76	80	66	60	50	6	98	104	69
5	138	136	79	92	96	72	46	40	7	108	113	73
6	208	168	91	90	93	80	118	75	11	117	124	86
8	178	167	113	111	117	107	67	50	6	135	142	112
9	137	125	82	84	94	76	53	31	6	97	107	79
Average	170	156	92	95	101	83	75	55	9	119	126	88
Differences	(1 less 2)	(2 less 3)		(1 less 2)	(2 less 3)		(1 less 2)	(2 less 3)		(1 less 2)	(2 less 3)	
Average†	14 ± 5	65 ± 7		-6 ± 1	18 ± 2		20 ± 5	47 ± 6		-7 ± 1	38 ± 3	
Range	2-40	43-76		3-10	10-27		6-43	25-69		5-10	28-55	
Significance of difference, p value‡	>0.05	>0.001		>0.01	>0.001		>0.01	>0.001		>0.001	>0.001	

*From recordings with simultaneous proximal aortic pressure.
†The numbers following the ± signs are the standard errors of the means.
‡Numbers with p values of less than 0.02 are considered to be statistically significant.

TABLE V. RATIO OF AORTIC PRESSURE ABOVE AND BELOW THE STRICTURE TO THE RADIAL ARTERIAL PRESSURE DURING REPAIR OF COARCTATION OF THE AORTA BEFORE RESECTION

PATIENT	PRESSURE RATIO							
	SYSTOLIC, AORTIC/RADIAL		DIASTOLIC, AORTIC/RADIAL		PULSE, AORTIC/RADIAL		MEAN, AORTIC/RADIAL	
	PROXIMAL AORTA	DISTAL AORTA	PROXIMAL AORTA	DISTAL AORTA	PROXIMAL AORTA	DISTAL AORTA	PROXIMAL AORTA	DISTAL AORTA
1	0.88	0.53	1.09	0.89	0.65	0.11	1.05	0.73
3	0.98	0.52	1.04	0.81	0.90	0.16	1.05	0.67
4	0.96	0.53	1.05	0.87	0.83	0.11	1.06	0.70
5	0.99	0.62	1.04	0.78	0.87	0.16	1.05	0.79
6	0.81	0.45	1.03	0.89	0.64	0.10	1.06	0.75
8	0.94	0.64	1.05	0.96	0.75	0.09	1.05	0.84
9	0.91	0.56	1.12	0.90	0.58	0.11	1.10	0.72
Average*	0.92 ± 0.02	0.55 ± 0.06	1.06 ± 0.03	0.87 ± 0.06	0.75 ± 0.13	0.12 ± 0.03	1.06 ± 0.02	0.74 ± 0.06

*The numbers following the ± signs are the standard errors of the mean.

TABLE VI. CHANGE IN AORTIC PRESSURE ABOVE AND BELOW THE STRICTURE AND IN RADIAL ARTERIAL PRESSURE ASSOCIATED WITH REPAIR OF COARCTATION OF THE AORTA

	PRESSURE, MM. OF MERCURY											
	SYSTOLIC			DIASTOLIC			PULSE			MEAN		
PATIENT	RADIAL	PROXIMAL AORTA	DISTAL AORTA	RADIAL	PROXIMAL AORTA	DISTAL AORTA	RADIAL	PROXIMAL AORTA	DISTAL AORTA	RADIAL	PROXIMAL AORTA	DISTAL AORTA
3	−15	−31	27	−7	−7	9	−8	−24	18	−12	−16	19
4	−24	−9	23	−4	3	5	−20	−12	18	−12	−3	14
5	3	2	44	1	3	27	2	−1	27	−1	1	36
6	−4	−7	44	5	7	17	−9	−14	27	7	3	38
8	−29	−22	3	−20	−12	−10	−9	−10	13	−23	−16	−3
9	−4	22	61	4	10	32	−8	12	29	9	16	45
Average	−12	−8	34	−4	1	13	−9	−8	22	−5	−3	25

and ranging from 3 to 36 mm. of mercury, and a small increase in the mean and diastolic pressures. There was an immediate decrease in the systolic, diastolic, pulse and mean pressures in the distal aorta averaging 5 mm. of mercury and ranging from 1 to 10 mm.

It is concluded that the root of the left subclavian artery carried a large portion of the blood destined to flow through the collateral circulation around the site of the coarctation.

After resection of the coarctation gradual opening of the completed end-to-end anastomosis of the aorta over a period of 31 (range 16 to 67) seconds in eight cases produced a decrease in systolic pressure in the radial artery of 22 (range 17 to 27) mm. of mercury, and an average decrease of 8 and of 11 mm. in the radial diastolic and mean pressures, respectively.

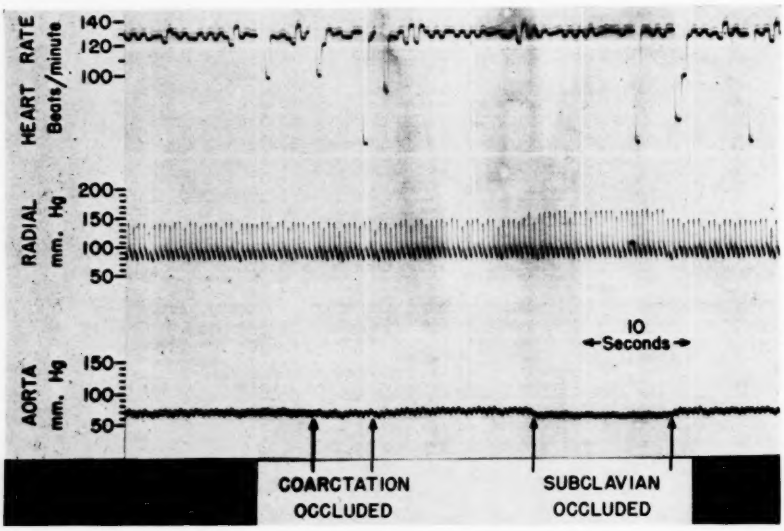

Fig. 4.—Effect of occluding the site of the stricture and the left subclavian artery on the blood pressure in the right radial artery and in the aorta *proximal* to the stricture during repair of coarctation of the aorta but before resection of the stricture. Occlusion of the site of the coarctation produced no apparent change in these pressures. Occlusion of the left subclavian artery produced an immediate increase in the systolic, diastolic pulse and mean pressure in the right radial artery of 16, 3, 13 and 4 mm. of mercury, respectively, and in the proximal aorta of 16, 4, 12 and 7 mm. of mercury respectively. The sudden transient changes in heart rate are artefacts associated with the surgical manipulation.

A comparison of the radial, proximal aortic, and distal aortic pressures and pulse conduction times recorded before and after completion of the anastomosis was made (Tables III and VI, Fig. 3). Owing to large variations in arterial pressure occurring throughout the course of the operation, caused by factors encountered in any major surgical procedure, the differences in radial and proximal aortic pressures recorded before and after completion of the anastomosis were not consistent (Table VI). The pressure in the aorta distal to the site of the coarctation, however, was, with one exception, increased after completion of the anastomosis. The average increases in systolic, diastolic, pulse and mean pressures were 34, 13, 22, and 25 mm. of mercury, respectively

(Table VI). In spite of the large nonspecific variations in the arterial pressure above the coarctation, the ratios of the distal aortic systolic, pulse or mean pressure to the respective radial artery pressures were increased in every instance (Table VII). The increase in the ratio of the distal aortic pulse pressure to the radial pulse pressure was the most striking, averaging 308 (155 to 570) per cent. The onset of the aortic pulse wave distal to the site of the coarctation, which initially occurred 0.02 second *after* the onset of the radial pulse, occurred 0.05 (range 0.04 to 0.07) second *before* the radial pulse when the anastomosis had been completed (Table III). The time interval from onset to peak (build-up time) of the distal aortic pulse was decreased from 0.18 (range 0.14 to 0.23) to 0.14 (range 0.11 to 0.19) second by completion of the anastomosis (Table III).

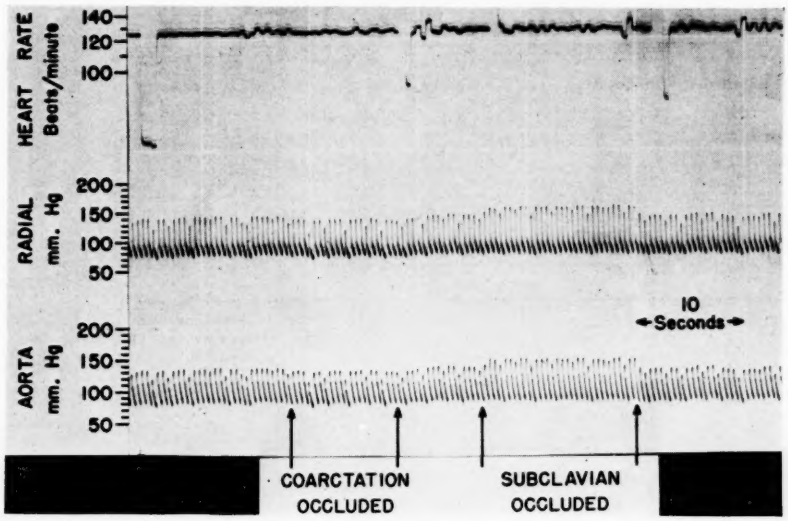

Fig. 5.—Effect of occluding the site of stricture and the left subclavian artery on the blood pressure in the right radial artery and in the aorta *distal* to the stricture during repair of coarctation of the aorta but before resection of the stricture. Occlusion of the site of the coarctation produced no apparent change in these pressures. Occlusion of the left subclavian artery produced an immediate increase in the systolic and pulse pressure of the right radial artery of 17 mm. of mercury and an immediate *decrease* in the systolic and mean pressures in the distal aorta of 6 mm. of mercury. The sudden transient changes in heart rate are artefacts associated with the surgical manipulation.

Direct studies of radial and femoral arterial pressures were carried out on these patients before and approximately two weeks after the surgical repair of the coarctation. A comparison of the preoperative and postoperative results (Tables VIII and IX) indicates that the average radial systolic pressure was reduced from the preoperative level of 197 (range 150 to 235) to 162 (range 127 to 193) mm. of mercury after operation (Table VIII). The femoral systolic pressure, on the other hand, was increased from 112 (range 91 to 135) to 149 (range 108 to 168) mm. of mercury after the operation (Table VIII). The calculated ratio of the femoral arterial pressure to the radial arterial pressure was increased in every instance. The mean increases in the systolic, pulse and mean pressure ratios approximately two weeks after the operation were 60, 210, and 35 per cent, respectively. The delay in onset of the femoral pulse was

TABLE VII. EFFECT OF REPAIR OF COARCTATION OF THE AORTA ON THE RATIO OF PRESSURE IN THE AORTA ABOVE AND BELOW THE STRICTURE TO THE PRESSURE IN THE RADIAL ARTERY RECORDED SIMULTANEOUSLY

	PRESSURE RATIO											
	SYSTOLIC, AORTIC/RADIAL				PULSE, AORTIC/RADIAL				MEAN, AORTIC/RADIAL			
	PROXIMAL AORTA		DISTAL AORTA		PROXIMAL AORTA		DISTAL AORTA		PROXIMAL AORTA		DISTAL AORTA	
PATIENT	AFTER REPAIR	CHANGE*	AFTER REPAIR	CHANGE*	AFTER REPAIR	CHANGE*	AFTER REPAIR	CHANGE*	AFTER REPAIR	CHANGE*	AFTER REPAIR	CHANGE*
2	0.85				0.62				1.07			
3	0.90	−0.08	0.73	0.21	0.71	−0.19	0.41	0.25	1.03	−0.02	0.94	0.27
4	1.08	0.12	0.84	0.31	0.95	0.12	0.59	0.48	1.17	0.11	0.98	0.28
5	0.98	−0.01	0.84	0.22	0.81	−0.06	0.47	0.31	1.07	0.02	0.99	0.20
6	0.79	−0.02	0.66	0.21	0.56	−0.08	0.35	0.25	1.02	−0.04	1.02	0.27
8	0.97	0.03	0.82	0.18	0.69	−0.06	0.36	0.27	1.13	0.08	1.02	0.18
9	1.11	0.20	1.02	0.46	0.96	0.38	0.74	0.63	1.16	0.06	1.11	0.39
Average	0.95	0.04	0.82	0.27	0.76	0.02	0.49	0.37	1.09	0.04	1.01	0.27

*Ratio before resection minus ratio after resection and repair of the coarctation.

TABLE VIII. EFFECT OF REPAIR OF COARCTATION OF THE AORTA ON THE INTRA-ARTERIAL RADIAL AND FEMORAL BLOOD PRESSURE

	PRESSURE, MM. OF MERCURY															
	SYSTOLIC				DIASTOLIC				PULSE				MEAN			
	RADIAL		FEMORAL		RADIAL		FEMORAL		RADIAL		FEMORAL		RADIAL		FEMORAL	
PATIENT	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
3	224	166	135	168	116	104	102	108	108	62	33	60	159	126	115	129
4	235	193	125	165	113	102	93	102	122	91	32	63	153	122	103	123
5	175	127	111	108	102	80	89	75	73	47	22	33	133	97	100	89
6	194	179	109	167	74	82	77	79	120	97	32	88	110	102	88	98
8	150	161	91	162	81	96	72	99	69	65	19	63	106	125	82	125
9	205	146	99	121	102	74	78	73	103	72	21	48	139	98	87	92
Average	197	162	112	149	98	90	85	89	99	72	27	59	133	112	96	109

TABLE IX. EFFECT OF REPAIR OF COARCTATION OF THE AORTA ON THE CONDUCTION TIME OF THE PULSE WAVE IN THE RADIAL AND FEMORAL ARTERIES

PATIENT	SECONDS FROM ONSET OF FEMORAL PULSE TO ONSET OF RADIAL PULSE			BUILD-UP TIME OF FEMORAL PULSE WAVE, SECONDS		
	BEFORE REPAIR	AFTER REPAIR	DIFFERENCE	BEFORE REPAIR	AFTER REPAIR	DIFFERENCE
3	-0.03	-0.01	0.02	0.24	0.16	-0.08
4	-0.04	0	0.04	0.19	0.13	-0.06
5	-0.04	-0.01	0.03	0.21	0.17	-0.04
6	-0.03	0	0.03	0.24	0.13	-0.11
8	-0.04	0	0.04	0.21	0.18	-0.03
9	-0.05	0.01	0.06	0.22	0.18	-0.04
Average	-0.04	0	0.04	0.22	0.16	-0.06

abolished after the operation and the build-up time of the femoral pulse was decreased in every instance, the average being from 0.22 (range 0.19 to 0.24) before operation to 0.16 (range 0.13 to 0.18) second postoperatively (Table IX).

In this series of cases, although the changes were in the same direction, a significant correlation could not be established between the degree of improvement in the circulatory dynamics in the distal aorta produced during the operation and the degree of improvement in circulatory dynamics found in the femoral artery two weeks after the operation had been completed.

COMMENT

The finding that the systolic and pulse pressures in the radial artery exceeded by 2 to 43 mm. of mercury the pressures generated by the same heartbeat in the proximal aorta is of interest. Actually, the mean gradient of pressure in these patients causing blood flow from the aorta to the radial artery was 7 (range 5 to 10) mm. of mercury. The temporary reversal of this gradient at systolic peak pressures has been explained as resulting from the conversion of the kinetic energy of flow to potential energy of pressure as the blood encounters increasing resistance to flow peripherally. Also, the presence of standing pressure waves in the arteries associated with the resonant characteristics of the arterial system may play a role in this phenomenon.[10]

The decrease in pressure in the proximal aorta which was observed as the completed anastomosis was slowly opened is of importance from the surgical point of view. Gross,[6] attributing the death of his first patient to a precipitous drop in blood pressure associated with opening the anastomosis, recommends that the completed anastomosis be opened gradually over a period of five to six minutes while the patient is placed in the Trendelenburg position and blood is transfused rapidly under pressure. In addition to this, Adams and coworkers[1] occluded the subclavian artery temporarily while the anastomosis was being opened. Certainly the average drop of 22 mm. of mercury in the systolic pressure and 11 mm. in the mean pressure noted in our 8 cases would not be considered alarming in spite of the fact that the average time spent opening the anastomosis was only thirty-one seconds. Other than occasionally speeding up the rate of the infusion, no special precautions were taken in these cases to maintain blood pressure.

Although there was an opening through the coaretation which in the resected specimen was about 1 mm. in diameter in each of the cases studied, the blood flow through this opening was, for practical purposes, negligible, since occlusion of the lumen produced no significant change in the pressure levels or in the ratios of the pressures above and below the stricture. Porjé[16] and Gupta and Wiggers[8] stated that the damping of the pressure pulse wave takes place when it passes through the stricture. Actually, it appears that the flow through the stricture does not contribute significantly to the pulse wave in the lower portion of the body and that this damping takes place when this pulse wave travels through the tortuous course of the collateral vessels.

In this regard it is to be noted that the applicability of results of hemodynamic studies in acutely produced coaretation to congenital coaretation can be questioned. In studies of acute coaretation the area of the aortic lumen may be reduced about 55 to 80 per cent to produce comparable changes in the femoral pulse contour and pressures[8] and there is little time for development of collateral circulation. In the usual clinical case, however, there is practically complete absence of the aortic lumen at the stricture and such extensive collateral circulation that reduction in blood flow to the lower part of the body is usually not demonstrable.[18]

In contrast to the lack of effect produced by occluding the site of the coaretation, occlusion of the left subclavian artery produced a fall in pressure in the distal aorta in spite of the fact that this maneuver caused an elevation in the arterial pressure proximal to the coaretation. These results indicate that there was a large blood flow through the left subclavian artery and that it was one of the major sources for the collateral flow around the coaretation. The advisability of using the subclavian artery to bridge the site of the aortic stricture is questionable since when this is done, the function that this vessel is already performing in conveying blood to the lower half of the body is sacrificed. This function was not present in the studies of acute coaretation in dogs reported by Gupta,[7] since a high degree of collateral circulation is not developed in this type of experimental coaretation. The possible beneficial effects of the subclavian type of anastomosis in clinical coaretation are for this reason almost certainly less[3, 9] than those reported in acutely produced coaretation.[7]

The results obtained in this study do not provide an answer to the important question concerning the exact nature of the hypertension in coaretation of the aorta. However, since opening of the completed anastomosis produced an immediate improvement in the circulatory dynamics (both the blood pressures above and below the stricture and the conduction time of the pulse wave), certainly mechanical hydrodynamic factors play an important role in the genesis of the hypertension which exists in this condition.

SUMMARY AND CONCLUSIONS

During nine operations for surgical repair of coaretation of the aorta in man, a mobile oscillographic camera was used to make simultaneous recordings of the electrocardiogram, respiration, heart rate, and direct blood pressure in the radial artery and in the aorta above and then below the site of stricture be-

fore and after resection and end-to-end anastomosis of the aorta. These studies indicated that (1) the average systolic and pulse pressures in the radial artery exceeded the simultaneously recorded pressures in the proximal aorta, while the radial diastolic and mean pressures averaged less than the pressures for the same heartbeat in the aorta proximal to the stricture; (2) systolic, diastolic, pulse and mean pressures in the aorta distal to the stricture averaged 41 mm. of mercury less than the simultaneously recorded radial arterial pressures; (3) occlusion of the site of the coarctation produced no change of pressure in the proximal or distal aorta; (4) occlusion of the left subclavian artery, however, produced an increase in proximal aortic pressure and, in spite of the increased central arterial pressure, a decrease in distal aortic pressure; and (5) opening the anastomosis produced an immediate decrease in proximal aortic pressure and an increase in distal aortic pressure. Thus it is evident that the mechanical factor of the stricture in coarctation of the aorta plays an important role in the genesis of the hypertension which exists in this condition.

Direct studies of radial and femoral arterial pressure carried out before and after operation on these patients likewise showed a change toward the normal state.

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A STUDY OF SERUM ELECTROLYTES (Na, K, Ca, P) IN PATIENTS WITH SEVERELY DECOMPENSATED PORTAL CIRRHOSIS OF THE LIVER

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IT HAD been previously noted by Eisenmenger and associates[1] that hyponatremia occurred in patients with cirrhosis of the liver. Studies of other serum electrolytes thus far have not been available. We were particularly interested in studying those electrolytes which are related to intermediary carbohydrate metabolism.

MATERIAL AND METHODS

Fourteen healthy subjects, the control series, and fifteen chronic alcoholic patients with portal cirrhosis were studied. The diagnosis in the latter group was confirmed by autopsy in five instances, and by liver biopsy in two. All patients were severely decompensated, presenting the picture of impending hepatic coma with dependent edema, ascites, and jaundice. Oliguria was present in eleven patients. Fever, leukocytosis, and mental confusion were common. Nine of the fifteen were disoriented. Neurologic findings included hyporeflexia (4), rigidity (2), positive Babinski response (8), and fecal and urinary incontinence (15). In each instance there was marked abnormality of all of the usual liver function tests, including the fractional serum bilirubin, cephalin cholesterol flocculation, thymol turbidity, zinc sulfate turbidity, alkaline phosphatase, total cholesterol with esters, plasma protein value including albumin-globulin ratio, twenty-four hour urine urobilinogen, and the prothrombin time.

Values of serum sodium and potassium were determined by means of the flame photometer; lithium was used as an internal standard. The determination of the serum calcium was done according to the method of Clark and Collip[2] and the inorganic phosphorus by the method of Fiske and Subbarow.[3] The pyruvic acid in the blood was analyzed by means of the Lu method[4] as modified by Friedmann and Haugen.[5] Initial blood samples were obtained within twenty-four hours of the patient's admission to the hospital.

RESULTS

The serum electrolyte values in the control series are in agrement with the values previously repirted in the literature (Table II).

In each patient with decompensated cirrhosis the initial values of all electrolytes studied were low, the differences being statistically significant (Table II). The mean serum sodium was 133.02 ± 5.14 meq. per liter. The serum potassium was 3.01 ± 0.56 meq. per liter. There were associated electrocardiographic abnormalities characteristic of hypopotassemia. The serum calcium was 4.21 ± 0.37 meq. per liter. The serum inorganic phosphorus was 1.21 ± 0.46 meq. per liter. The blood pyruvic acid was elevated in each instance, the mean value being 1.72 ± 0.35 mg. per cent, which is in agreement with a previous report[6] (Table I).

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TABLE I. DECOMPENSATED CIRRHOSIS

PATIENT	PYRUVIC ACID (MG. %)	NA (MEQ./L.)	K (MEQ./L.)	CA (MEQ./L.)	P (MEQ./L.)
E. M.	1.53	133.00	3.46	4.21	1.11
R. C.	1.91	130.40	3.30		
S. H.	1.34	127.40	3.60		
R. H.	1.69	139.00	2.93	3.60	0.61
A. T.	1.62	125.20	2.80	3.85	0.72
R. K.	1.42	142.20	3.26		
H. R.	1.51	141.30	2.60		1.44
C. S.	1.76	135.70	2.05	4.05	0.92
B. R.	1.48	133.90	2.36		
B. C.	1.36	129.00	3.60	4.20	1.06
A. W.	1.99	128.70	2.58	4.15	1.22
J. L.	1.82	133.90	2.36	4.29	2.26
M. C.		129.60	3.81	4.68	1.40
E. M.	2.01	133.00	3.46	4.21	1.11
J. H.	2.66			4.90	1.51
$\bar{X}$	1.72 ± 0.35	133.02 ± 5.14	3.01 ± 0.56	4.21 ± 0.37	1.21 ± 0.46

DISCUSSION

In addition to the dietary management,* an attempt was made to correct the serum electrolytes by means of intravenous sodium chloride and/or potassium chloride. The low serum values of these ions had been noted before the institution of therapy. A rapid clinical improvement was apparent as soon as the dietary intake was adequate. The correction of these ions, either by dietary or parenteral means, is necessary for recovery and proper therapeutic management. None of these patients went into anticipated hepatic coma, but all showed improvement so that thirteen were able to be discharged. Five have subsequently died of other complications, including massive hemorrhage from a ruptured esophageal varix (3) and cerebrovascular hemorrhage (2).

The explanation of the low values of these serum electrolytes is not definitely known. In none of these patients had there been loss of ascitic fluid by abdominal paracentesis. Correction of the serum sodium and potassium to normal values was not possible in some patients. In two patients the amount of sodium and potassium administered was almost entirely excreted in the urine without any effect on the serum values. A disturbed renal control of electrolytes as does occur in primary nonrenal disease may be an explanation; this has been noted in tuberculous meningitis,[7] pneumonia, peritonitis,[8] and pulmonary tuberculosis.[9]

TABLE II. STATISTICAL ANALYSIS OF BLOOD VALUES IN DECOMPENSATED CIRRHOSIS

ELECTROLYTE AND ORGANIC ACID	NO.	CONTROL (MEQ./L.)	NO.	DECOMPENSATED CIRRHOSIS (MEQ./L.)	t	p
Sodium	10	144.55 ± 5.44	14	133.02 ± 5.14	2.43	<0.05
Potassium	11	5.16 ± 0.67	14	3.01 ± 0.56	5.04	<0.01
Calcium	13	5.03 ± 0.26	10	4.21 ± 0.37	6.26	<0.01
Phosphorus	11	2.45 ± 0.36	11	1.21 ± 0.46	7.19	<0.01
Pyruvic acid	14	(mg. %) 1.06 ± 0.16	14	(mg. %) 1.72 ± 0.35	6.40	<0.01

*The approximate amounts of the ions in the diets were calcium 1.9 to 2.0 Gm., potassium 6.6 to 7.0 Gm., and phosphorus 2.5 to 3.0 Gm. per twenty-four hours.

These patients had all been subject to inadequate nutritional intake for periods of from four to six months. It is possible that this may in part account for the low values of serum sodium and potassium. The normal adult daily ingests and excretes from 3 to 4 Gm. of potassium (75 to 100 meq.).[10, 11] Fasting adults receiving water may excrete from 1 to 3 Gm. (25 to 75 meq.) of potassium daily.[12, 13] The daily loss of potassium of individuals on a diet calorically adequate but practically free of potassium is of the same order of about 2 Gm. (49 to 53 meq.).[14]

An inadequate diet may also be a factor in producing low values of serum phosphorus and calcium. The normal daily intake and excretion of phosphorus is from 0.6 to 2.8 Gm. (20 to 90 meq.). Fasting adults receiving water continued a daily urinary loss of from 0.58 to 1.27 Gm. (20 to 40 meq.) of phosphorus.[11]

The hypocalcemia and hypophosphatemia may also be related to steatorrhea. Gross and associates[15, 16] found the fecal fat to be increased from two- to three-fold in patients with cirrhosis. In steatorrhea the serum phosphorus and calcium have been found to be low due to the formation of soaps, increased gastrointestinal motility, and decreased gastric acidity.[17, 18] There also was noted a normal excretion of phosphorus in the urine in spite of the low serum phosphorus in chronic steatorrhea.

SUMMARY

Serum electrolytes (Na, K, Ca, P) have been studied in fifteen alcoholic patients with severely decompensated portal cirrhosis who presented the picture of impending hepatic coma. All electrolytes were found to be abnormally low in each instance before treatment.

The present study indicates that the low serum electrolytes are primarily related to a dietary deficiency. Other mechanisms, such as a disturbed renal control of electrolytes and steatorrhea, should also be considered.

Correction of this electrolyte depletion, as of other deficiencies, is of importance in the proper treatment of these cases.

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ESTIMATION OF ASCITIC FLUID VOLUMES

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DURING investigations on body water distribution in cases of cirrhosis with ascites, it was necessary to know the volume of ascitic fluid present. Ascitic fluid volumes can be estimated directly by paracentesis or indirectly by determining the extent of dilution of a solute injected intraperitoneally. Vital red,[1] Evans blue,[1, 2] and subtosan (polyvinyl-pyrrolidone[3]) have been used to measure ascitic fluid volumes by the indirect method. In only one paper were ascitic fluid volumes estimated by the simultaneous injection of more than one solute.[1]

Since ascitic fluid is not a stagnant pool, but is in relative dynamic equilibrium with plasma water,[4, 5] the rate of disappearance or clearance of any solute from the peritoneal cavity will be a function of many factors. If the decrement in concentration in relation to time is linear, one may then assume that the substance disappears at a rate proportional to its concentration. Extrapolation back to "zero time" will then yield the concentration occurring at instantaneous equilibration prior to loss from any route. The volume of distribution of a solute in ascitic fluid may then be computed as follows:

$$\text{Ascitic fluid volume (ml.)} = \frac{\text{amount of material injected (mg.)}}{\text{concentration at instantaneous equilibration of solute in ascitic fluid (mg./ml.)}}$$

This study was undertaken to measure ascitic fluid volumes by the indirect dilution method employing various solutes. In addition, the investigation was designed to evaluate the completeness of removal of fluid by paracentesis procedures.

METHODS

The following solutes were used: (1) bromsulfalein (BSP) was chosen because it was readily available and easily measured. Five milliliters (250 mg.) were injected intraperitoneally at a slow rate with the needle well within the peritoneal cavity to avoid irritation by the solute. Determinations of BSP in ascitic fluid samples were made with the Coleman spectrophotometer by the method of Gaebler.[6] (2) Radioactive iodo-albumin

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solution containing I^{131} and 5 mg. of protein per milliliter at a pH of 6.0 Ionic I^{131} activity was less than 1 per cent. Approximately 25 μc diluted to 20 ml. with 0.9 per cent sodium chloride solution were injected. Determination of activity in samples were made with GM mica window tube, Model D-32. (3) Para aminohippurate (PAH). Twenty milliliters of a 5 per cent solution were injected. PAH was determined by the method described by Goldring and Chasis.[7] All determinations were run in duplicate. Ascitic fluid blank specimens were run in all cases.

The patients studied had the classical manifestations of Laennec's cirrhosis with the exception of one patient with chronic cardiac failure. The patients selected either had not had a previous abdominal paracentesis or had not had one performed in the previous four weeks. Tests were repeated on three patients.

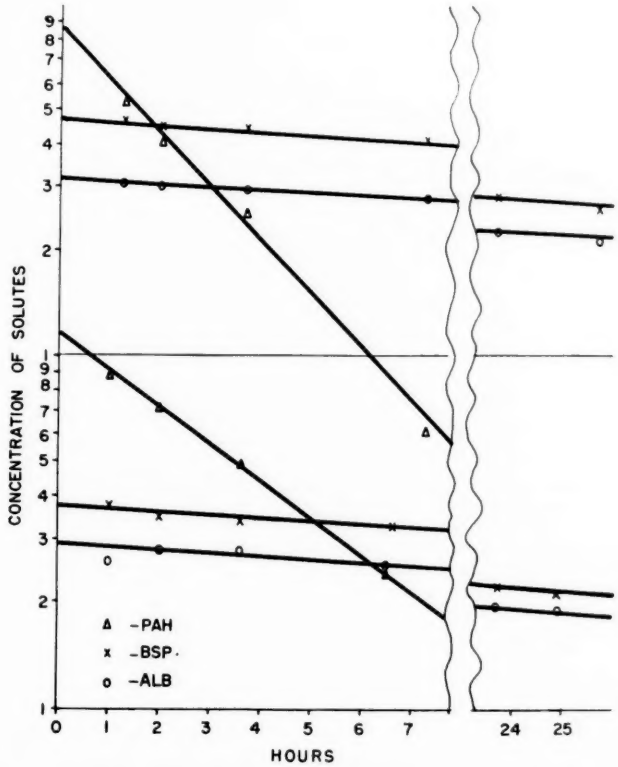

Fig. 1.—Time requirements for equilibration of BSP in ascitic fluid. The logarithm of the BSP concentration is plotted against time, following the intraperitoneal injection of 250 mg. at 0 time. Regression lines on five patients are shown. The sixty-minute sample deviated markedly from the regression, indicating inadequate mixing.

A sample of ascitic fluid, approximately equal in quantity to the volume to be injected, was withdrawn through a 20-gauge needle. The test materials were then injected through the same needle. After injection, the needle was with drawn, the abdomen ballotted, and the patient's position shifted frequently over a fifteen-minute period. A 17-gauge needle was then inserted intraperitoneally in an area 15 to 25 cm. away from the site of the previous injection, and a fine nylon catheter[8] was threaded through the needle into the peritoneal cavity. The 17-gauge needle was then removed and the nylon catheter taped to the abdominal wall. A 24-gauge hypodermic needle was inserted into the end of the catheter and ascitic fluid

samples were periodically withdrawn by a syringe. During the intervals between the drawing of samples, a Luer-Lok glass adapter sealed by a serum cap was fitted to the needle hub and filled with a heparin-saline solution.

Paracenteses were performed in the routine manner by the insertion of a trocar through the linea alba midway between the pubis and umbilicus, with the patient in the sitting position. Manual compression of the abdomen and changes in the patient's position were effected to assist complete collection of ascitic fluid. In an occasional patient where incomplete drainage was suspected, the trocar was reinserted lateral to the midline.

RESULTS

No toxic reactions were noted following the intraperitoneal injection of BSP. Fig. 1 illustrates the rates of diffusion of BSP from ascitic fluid in a series of five patients. Approximately two hours were required for equilibration of BSP in the peritoneal fluid. Although only the two- and four-hour ascitic fluid concentrations are plotted in Fig. 1, the logarithms of serial BSP concentrations up to twenty-four hours following injection were all a linear function of time. Exemplary time-concentration regressions in ascitic fluid with the simultaneous use of PAH, BSP, and radioactive albumin in two patients are presented in Fig. 2. Extrapolation of these lines back to zero time yield the theoretic concentration at instantaneous equilibration. Dividing the amount of the solute injected by the ascitic fluid concentration at zero time, one obtains the volume of distribution of the particular solute used. Data on ascitic fluid volumes in a series of five patients as determined by the simultaneous use of BSP, radioactive albumin, and PAH are presented in Table I. It can be seen that the BSP and radioactive albumin volume agreed closely in four of the five patients. The PAH volumes were definitely higher than BSP volumes in three of five patients, and agreed with radioactive albumin in four of five patients. Following the indirect measurement of ascitic fluid volume in five patients, a paracentesis was performed to obtain a direct measure of the ascitic fluid volume. From Table II, it can be seen that the two volumes, the indirect ascitic fluid volume and that obtained by direct paracentesis, compare favorably with a mean difference of 440 milliliters.

DISCUSSION

In the measurement of body water partition in patients with cirrhosis and ascites, it is of importance to know the volume of ascitic fluid present. The data

TABLE I. COMPARATIVE ASCITIC FLUID VOLUMES AS DETERMINED BY THE VOLUMES OF DISTRIBUTION BSP, RADIOACTIVE ALBUMIN, AND PAH

PATIENT	VOLUME OF ASCITIC FLUID (LITERS)			DIFFERENCE BETWEEN BSP AND PAH VOLUMES	
	BSP (I)	RADIOACTIVE ALBUMIN	PAH (II)	I-II (LITERS)	PER CENT*
1. Ga., II	5.27	5.31	5.43	−0.16	3.0
2. Su., II	11.30	13.48	13.35	−2.05	18.2
3. Rot.	7.90	7.92	8.06	−0.16	2.0
4. Roe.	7.57	8.37	8.96	−1.39	18.4
5. Me.	6.50	6.88	8.98	−2.48	38.3
Mean	7.71	8.39	8.97	−1.25†	16.0

*Per cent $= \frac{I - II}{I} \times 100.$

†Standard deviation = 1.07; P = 0.08.

TABLE II. COMPARISON OF ASCITIC FLUID VOLUMES AS MEASURED BY BSP AND/OR RADIOACTIVE ALBUMIN WITH THOSE OBTAINED BY PARACENTESIS

PATIENT	INDIRECT ESTIMATION OF ASCITIC FLUID VOLUME (LITERS)		PARACENTESIS VOLUME (LITERS) II	DIFFERENCE BETWEEN BSP AND PARACENTESIS VOLUMES	
	BSP (I)	RADIOACTIVE ALBUMIN		I-II (LITERS)	PER CENT*
Me.	6.50	6.88	6.30	+0.20	3.1
Su., I	16.13	16.58	16.00	+0.13	8.1
Kr.	5.97	5.89	5.87	+0.10	1.7
Ga., I	4.81	3.87	4.80	+0.01	0.0
Ja.	9.00	9.77	7.25	+1.75	19.5
Mean	8.48	8.60	8.05	+0.44[2]	6.5

Standard deviation = 0.43; P = 0.3.

*Per cent = $\frac{I - II}{I} \times 100$.

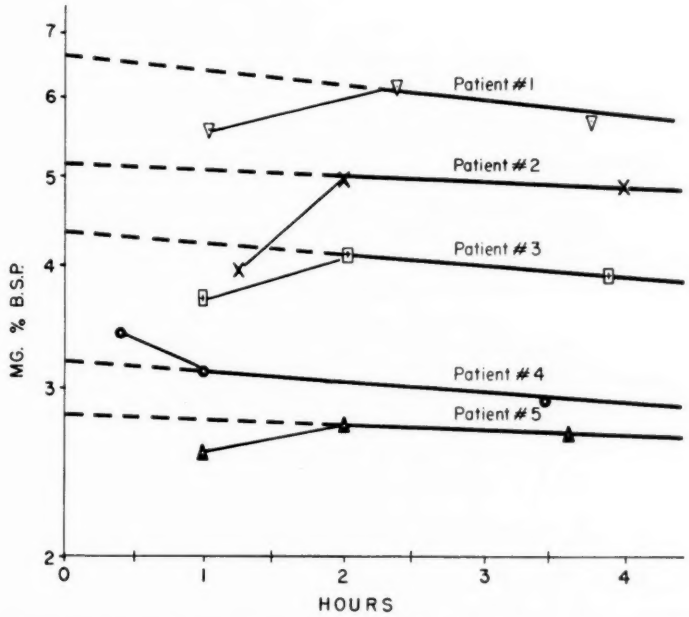

Fig. 2.—Time-concentration regressions for PAH (mg. per cent), BSP (mg. per cent), and radio-active albumin (counts / ml. / mm.) in ascitic fluid. The logarithm of the ascitic fluid concentration is plotted against time. Data on two patients are presented.

presented in this paper indicate that ascitic fluid volumes can be indirectly measured by determining the degree of dilution of various solutes injected directly into the peritoneal cavity. In contrast to the findings of Mankin and associates who allowed thirty to sixty minutes for mixing of T-1824, upwards to one hundred twenty minutes were required for equilibration of BSP. Following equilibration, relatively close correlation was noted between the volumes of distribution of three solutes injected simultaneously. These data would seem to support the accuracy of the indirect volume measurement employing any one of the three solutes used. Further evidence of the relative accuracy of the indirect method of measuring ascitic fluid volume is the volume of fluid obtained

by direct paracentesis. Volumes of ascitic fluid obtained by paracentesis agreed closely with those obtained by the solute-dilution technique. The BSP volumes exceeded the paracentesis yield by a mean of 440 ml., which may have been residual fluid lying in the pelvic gutters. These findings were in accord with those of Mankin and co-workers, who reported a mean discrepancy of 375 ml. between paracentesis yields and T-1824 volumes. In contrast to our experiences, Warter and associates reported that subtosan volumes exceeded paracentesis yields by a mean of 3.1 liters in four patients. It should be noted that the patients studied in our series either had had no previous paracenteses or had not been tapped in the previous month. Warter and associates make no mention of similar information in their report. Intraperitoneal inflammation associated with recent taps or due to other causes may result in loculation of fluid which could conceivably affect the accuracy of paracentesis volumes more markedly than that of solute-dilution volumes where homogeneous distribution might still occur with time.

It is of interest to note that the clearance of BSP and radioactive albumin from ascitic fluid are comparable. Among other factors, the particle size or molecular weight are important in the rate at which any solute diffuses out of the peritoneal cavity, that is, the smaller the particle the more rapid the diffusion. The more rapid diffusion of PAH (molecular weight, 216), compared with radioactive albumin (molecular weight, 69,000), is in line with this thesis. The rate of diffusion of BSP (molecular weight, 838), closely parallels that of radioactive albumin, and is in accord with evidence that BSP is adsorbed by proteins.[10]

For routine clinical use, ascitic fluid volumes can be estimated by simply injecting 5.0 ml. of BSP solution intraperitoneally, and drawing a single ascitic fluid sample after two to three hours. The volume obtained by this procedure will fall within 10 per cent of that measured by the more accurate serial sampling technique.

SUMMARY

1. Close correlation was found in the volumes of distribution of bromsulfalein and radioactive albumin, and between para-aminohippurate and radioactive albumin in ascitic fluid of four out of five patients with cirrhosis. Although para-aminohippurate volumes agreed closely with bromsulfonphthalein in only two of five patients, the mean difference between the two volumes was not statistically significant.

2. Ascitic fluid volumes, as measured by means of the degree of dilution of various solutes injected intraperitoneally, checked within a mean of 440 c.c. with volumes obtained by paracentesis in the patients studied.

3. For routine clinical purposes, bromsulfalein may be used to estimate ascitic fluid volumes by the indirect dilution method.

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SIMULTANEOUS DETERMINATION OF TOTAL BODY WATER BY ANTIPYRINE AND DEUTERIUM OXIDE; EVALUATION OF THE METHODS ON EDEMATOUS SUBJECTS

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INTRODUCTION

TWO of the more useful methods for the measurement of total water content of the body are those utilizing antipyrine and deuterium oxide. The measurements are based upon the dilution of these substances produced, after a suitable time has elapsed for their equilibration throughout the organism. Heavy water is considered to diffuse throughout the body as does ordinary water. The same has been shown to be true for antipyrine. Since antipyrine is transformed or metabolized at a constant rate, several plasma samples are required, and the line produced by a semi-logarithmic plot of the values obtained is extrapolated back to the time of injection.[1]

Soberman and his associates, in their description of the procedure for antipyrine, reported simultaneous studies on eight normal subjects, with good agreement between the two methods. In seven edematous subjects, the antipyrine volumes averaged 2.7 L. (0.9 to 5.1 L.) less than the deuterium volumes. The degree of edema was not stated. They also reported fair correlation in the distribution of antipyrine between plasma and serous cavity fluid in eight instances in from seven to twelve hours after injection.

The purpose of this study was to attempt further evaluation of the usefulness of the two methods in edematous patients by: (1) further comparison of simultaneous observations, (2) correlation of derived volume losses with weight losses after the clearing of edema, and (3) serial comparison of concentrations of the agents in blood, interstitial fluid, and serous cavity fluid to compare rates of diffusion.

MATERIALS AND METHODS

The subjects were patients with edema of varying origin who were admitted to the Metabolic Unit of the Deaconess Hospital. Fifty-five grams of deuterium oxide and 1.5 Gm. of antipyrine were introduced by vein to fasting subjects on the morning of the first full day of hospitalization. Samples of venous blood were drawn in heparinized syringes at intervals varying from three to twenty hours after injection. Approximately 2 ml. of whole blood were

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TABLE I. SIMULTANEOUS ANTIPYRINE AND DEUTERIUM VOLUMES IN SUBJECTS WITH EDEMA

SUBJECT	EDEMA WEIGHT LOSS (KG.)	ANTIPYRINE		DEUTERIUM		DIFF. LITERS	DIAGNOSIS
		LITERS	% BODY WEIGHT	LITERS	% BODY WEIGHT		
R. E.	1.1	40.5	62.3	42.2	64.8	−1.7	Rheumatic heart disease
G. D.	3.0	40.1	60.3	45.1	67.8	−5.0	Rheumatic heart disease
E. G.	3.4	41.7	46.7	46.3	51.4	−4.6	Arteriosclerotic heart disease with hypertension
R. S.	5.0	50.2	60.3	52.6	63.0	−2.4	Chronic nephritis with hypertension
H. L.	5.1	55.6	50.5	62.5	56.9	−6.9	Arteriosclerotic heart disease
G. G.	6.1	31.4	57.0	38.8	70.5	−7.4	Rheumatic heart disease
C. M.	6.8	31.0	60.6	34.7	68.0	−3.7	Arteriosclerotic heart disease
F. T.	7.1	26.6	55.2	32.0	66.4	−5.4	Arteriosclerotic heart disease with hypertension
D. H.	8.1	31.4	38.9	41.3	51.2	−9.9	Rheumatic heart disease
F. O.	8.4	44.6	68.3	52.1	80.0	−7.5	Chronic pyelonephrosis with hypertension
S. B.	10.7	33.1	50.0	40.3	61.0	−7.2	Arteriosclerotic heart disease
J. M.	10.9	36.2	60.4	39.7	66.3	−3.5	Chronic glomerulonephrosis (nephrotic stage)
G. C.	12.0	37.7	47.5	50.0	63.0	−12.3	Arteriosclerotic heart disease
R. M.	12.2	31.0	52.0	39.1	65.6	−8.1	Rheumatic heart disease
H. L.	12.4	61.0	51.6	62.5	53.0	−1.2	Arteriosclerotic heart disease
M. W.	12.8	34.9	59.5	41.6	70.8	−6.7	Rheumatic heart disease
T. L.	13.3	44.6	50.5	50.0	56.6	−5.4	Arteriosclerotic heart disease
N. G.	14.0	36.4	48.4	43.8	58.3	−7.4	Arteriosclerotic heart disease
K. G.	15.9	50.7	66.0	51.6	67.0	−0.9	Arteriosclerotic heart disease —diabetes
W. D.	17.2	42.3	52.6	57.5	71.3	−15.2	Arteriosclerotic heart disease —cirrhosis
C. M.	24.2	55.8	55.3	72.5	70.5	−16.7	Arteriosclerotic heart disease
E. O.	39.7	58.2	56.8	66.7	65.2	−8.5	Arteriosclerotic heart disease
R. L.	Massive	35.2	36.0	39.7	40.6	−4.5	Arteriosclerotic heart disease —diabetes
E. B.	Massive	45.0	62.2	60.2	83.2	−15.2	Arteriosclerotic heart disease
E. L.	Massive	40.2	56.9	44.6	63.0	−4.4	Cor pulmonale
J. N.	Massive	41.1	57.0	52.1	72.3	−10.0	Arteriosclerotic heart disease
L. M.	Massive	35.1	59.0	42.2	71.0	−7.1	Rheumatic heart disease
I. S.	Massive	27.5	42.5	31.6	48.9	−4.1	Arteriosclerotic heart disease —diabetes
F. T.	Moderate	38.3	58.4	49.0	74.9	−10.7	Chronic glomerulonephrosis with hypertension—uremia
Average Values		40.6	54.6	47.7	64.2	−7.1	

apportioned for deuterium analysis, and the remainder was centrifuged to obtain plasma for antipyrine analysis. Except for breakfast, meals were allowed at the usual times, and fluid intake was not altered from the usual.

Deuterium concentrations were determined by a falling drop method in a constant temperature water bath according to the principles outlined by Cohn.[2] Water from the blood samples was obtained by lyophylization, and trapping of the water vapor as ice in a tube surrounded by a freezing mixture. Antipyrine concentrations were determined as described by Brodie and associates,[3] utilizing an ultraviolet attachment for the Beckman DU Spectrophotometer.

Interstitial fluid was obtained free of blood from a dependent extremity using needles patterned after those described by Burch.[4] Serous cavity fluid was obtained by simple aspiration. Water content of interstitial and serous cavity fluid was determined by oven drying to a constant weight of a tared amount of fresh material. Patients were freed of their edema by clinical methods previously reported.[5]

Twenty-eight subjects are included in the study. Sixteen had arteriosclerotic cardiovascular disease, seven had rheumatic heart disease, four had chronic nephritis, and one had

TABLE II. SIMULTANEOUS VOLUME MEASUREMENTS BEFORE AND AFTER REMOVAL OF EDEMA

SUBJECT	ANTIPYRINE VOLUME			DEUTERIUM VOLUME			KG. OBSERVED WEIGHT LOSS
	BEFORE	AFTER	DIFF.	BEFORE	AFTER	DIFF.	
E. G.	41.7	41.8	+0.1	46.3	45.4	0.9	3.2
R. S.	50.2	43.0	7.2	52.6	47.6	5.0	5.0
H. L.	55.6	51.0	4.6	62.5	60.2	2.3	5.1
G. G.	31.4	27.8	3.6	38.8	32.6	6.2	6.1
C. M.	31.0	24.2	6.8	34.7	31.4	3.3	6.8
F. T.	26.6	21.7	4.9	32.0	29.6	2.4	7.1
D. H.	31.4	30.7	0.7	41.3	34.2	7.1	8.1
F. O.	44.6	33.2	11.4	52.1	43.1	9.0	8.4
S. B.	33.1	26.9	6.2	40.3	31.0	9.3	10.7
J. M.	36.2	26.5	9.7	39.7	31.2	8.5	10.9
G. C.	37.7	28.7	9.0	50.0	39.1	10.9	12.0
R. M.	31.0	25.7	5.3	39.1	27.3	11.8	12.2
H. L.	61.0	56.4	4.6	62.5	56.2	6.3	12.4
M. W.	34.9	24.1	10.8	41.6	31.4	10.2	12.8
T. L.	44.6	32.1	12.5	50.0	41.6	8.4	13.3
N. G.	36.4	26.6	9.8	43.8	33.4	10.4	14.0
K. G.	50.7	37.5	13.2	51.6	39.0	12.6	15.9
W. D.	42.3	32.8	9.5	57.5	44.2	13.3	17.2
C. M.	55.8	40.6	15.2	72.5	51.0	21.5	24.2
E. O.	58.2	31.2	27.0	66.7	36.5	30.2	39.7
Average Value	41.7	33.1	8.6	48.8	39.3	9.5	12.25

chronic cor pulmonale. In one patient cirrhosis of the liver, and in three patients diabetes was coexistent. One patient was studied in two episodes of congestive heart failure.

RESULTS

1. The results of simultaneous measurements of total body water in edematous subjects in twenty-nine instances are summarized in Table I. The average antipyrine volume for the group was 40.6 L., or 54.6 per cent of body weight. The average deuterium volume was 47.7 L., or 64.2 per cent of body weight. In no instance did an antipyrine volume exceed a deuterium oxide volume.

In the group of ten subjects whose subsequent weight loss was less than 10 kg., the average antipyrine-deuterium volume difference was –5.4 L. (1.7 to 9.9 L.). In the group of ten whose weight loss was between 10 and 20 kg., the average difference was –6.8 L. (0.9 to 15.2 L.). In the group of three whose weight loss was over 20 kg. (including subject R. L.), the average difference was –9.9 L. (4.5 to 16.7 L.). This suggests the possibility of a difference in the rate of diffusion of the two agents throughout the body, which is increased in the massively edematous subject.

2. In twenty instances volumes were determined simultaneously with the two agents on the edematous patients, and were repeated after the edema was removed. The correlation of the volume loss as derived from the two methods with the observed weight loss is presented in Table II. The time intervals between determinations averaged eighteen days (six to sixty-eight days).

Despite the discrepancy shown between the absolute volumes as determined simultaneously, the derived volume changes in these "before and after" studies were not too divergent. For antipyrine the mean volume loss was 8.6 L., and for deuterium it was 9.5 L. The observed average weight loss for the group was

TABLE III. COMPARISON OF RATES OF EQUILIBRATION OF ANTIPYRINE AND DEUTERIUM IN EDEMATOUS SUBJECTS

	ANTIPYRINE			DEUTERIUM			
SUBJECT	TISSUE FLUID (HR.)	CHEST FLUID (HR.)	ASCITIC FLUID (HR.)	TISSUE FLUID (HR.)	CHEST FLUID (HR.)	ASCITIC FLUID (HR.)	DEGREE OF EDEMA
R. E.		0—7			+—5		1.1 kg.
C. M.		0—8			+—6		6.8 kg.
R. H.	0— 8.5	0—8.5		+— 8.5	+—8.5		9.6 kg.
F. P.	0— 9.5	0—9.5		0— 9.5	+—9.5		Massive
E. G.	+— 4			+— 3			3.1 kg.
H. L.	0— 7			+— 6			5.1 kg.
F. O.	+— 6			+— 3			8.4 kg.
E. L.	0—20			+—20			Massive
J. N	0— 8			+— 8			Massive
G. C.	0— 9			+— 9			12.1 kg.
R. L.	0— 6			0— 6			Massive
E. B.	0— 7		0—7	+— 6		+—4	Massive
C. M.	0— 9		0—9	0— 9		+—4	24.2 kg.
T. L.			+—4			+—4	13.3 kg.
W. D.		0—8	0—8		+—7	0—8	17.2 kg.
Totals	11	5	4	11	5	4	
Number in whom equilibrium was reached	2	0	1	8	5	3	

+ – Denotes that equilibration was reached, followed by the time in hours.
0 – Denotes equilibration was not reached, followed by the duration of the study in hours.

12.25 kg. Nitrogen balances were not done, but undoubtedly many of the subjects were in negative balance and in an eighteen-day period could conceivably lose as much as 2 kg. of tissue weight.

3. The observation that the antipyrine space averages somewhat lower than the deuterium space has not been explained. Soberman suggests that the difference may be due to failure to correct for small amounts of antipyrine that become protein bound.[1] However, a similar error may be present with deuterium oxide, for it has been suggested that some heavy hydrogen may exchange with available bound ordinary hydrogen in the body.[6] We have attempted to approach the problem by following the rates of diffusion of the materials by simultaneous serial determination of their concentrations in blood, interstitial fluid, ascitic fluid and/or pleural fluid. At equilibrium throughout the body, the respective concentrations should be equal in the various body fluids studied. The results are summarized in Table III. Equilibrium was assumed reached when the corresponding values agreed within 4 per cent of each other.

It is apparent that the diffusion rate for deuterium is more rapid than for antipyrine, particularly into serous cavity effusions, as evidenced by the fact that with antipyrine equilibration was reached in one of nine instances, while with deuterium it was reached in eight of nine instances. In interstitial fluid equilibration was reached with antipyrine in two of eleven instances, while with deuterium it was reached in eight of twelve instances. The rate of diffusion appears to correlate roughly with the degree of edema present.

DISCUSSION

When antipyrine and deuterium total body water volumes are determined simultaneously in edematous patients, the antipyrine volumes are consistently

smaller than the deuterium volumes. The degree of variation is roughly parallel to the degree of edema present. Yet when the two methods are utilized simultaneously to determine *changes* in total body water as produced by removal of edema, the derived volume changes agree within 10 per cent, though the antipyrine changes were consistently smaller. These derived volume changes agree well with the observed weight loss (edema) in the same subjects.

The smaller absolute volumes and the smaller changes in volume as edema is lost as derived from the antipyrine method are based to a considerable extent upon a slower rate of diffusion of antipyrine as compared to deuterium. It would appear that both methods are useful where edema is present in moderate degree and where long periods of time are allowed for antipyrine diffusion. In the presence of massive edema and/or large accumulations of fluid in serous cavities, the deuterium method is more accurate. However, in such cases a long period of time is necessary before equilibration occurs.

SUMMARY

Comparative results of the antipyrine and deuterium oxide methods for determination of total body water in edematous subjects has been presented.

In such subjects the deuterium volume is uniformly greater than the antipyrine volume. When these methods are used to measure changes in total body water as edema is removed, they both reflect changes which agree well with observed weight losses. Losses reflected by changes in deuterium volumes however, were consistently somewhat greater than those reflected by antipyrine losses and more nearly approached the observed weight changes.

Data are presented which demonstrate that antipyrine diffusion throughout the body is slower than deuterium diffusion.

In the presence of massive edema with serous cavity effusion, the deuterium method is considered more accurate, but care must be taken to allow considerable time for equilibration to occur.

The constant temperature water bath and temperature control mechanism used in the study for deuterium analyses were constructed and furnished by the Holter Research Foundation, Helena, Mont.

The antipyrine used in these studies was furnished by the Eli Lilly Company, who also prepared the deuterium oxide for intravenous use.

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URINE-BLOOD RATIOS OF DEUTERIUM OXIDE IN MAN

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ONE of the more useful procedures for determination of total body water has been that utilizing deuterium oxide. Theoretically this material is an "ideal" agent for the purpose since it is an isotope of the normal constituent water, and rapidly equilibrates throughout the entire body.[1]

As one phase of evaluating the method, it is desirable to determine whether the functioning renal tubule is capable of distinguishing between water and its isotopic form deuterium oxide. If no distinction is made, deuterium oxide will be reabsorbed by the tubule in the same ratio to water as is present in the glomerular filtrate. In this circumstance, at any given time, the blood deuterium oxide to water ratio would equal the urine deuterium oxide to water ratio.

MATERIALS AND METHODS

Fifty-five grams of deuterium oxide were given intravenously or orally to fasting subjects with edema, without edema, and to normal individuals. Water was allowed ad libitum in all instances but two, in one of which it was restricted, and in the other it was forced. Subjects, while standing,voided or were catheterized five to ten minutes before collection of blood, and the urine produced in the succeeding ten to twenty minutes was collected for analysis. Two milliliters of venous blood were withdrawn in a heparinized syringe at the midpoint in time of the urine collections. Samples were obtained at hourly intervals for varying lengths of time. They were shell frozen in a flask immersed in a mixture of dry ice and ethylene glycol and lyophylized, the water vapor being collected as ice in a trap surrounded by a similar freezing mixture.

Deuterium oxide to water ratios (expressed as per cent) were determined by falling drop in a constant temperature water bath based upon principles outlined by Cohn.[2]

RESULTS AND DISCUSSION

The results are summarized in Table I.

At an average concentration of 0.100 per cent deuterium oxide in water solution, the error of the method is 1 to 2 per cent. When the results are

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expressed as a urine/blood ratio, this error allows a deviation of 1.00, ± 0.04. In the time intervals greater than two hours after administration of deuterium oxide, this range was exceeded in seven of sixty-four instances (plus 0.07 to minus 0.06). In the first two hours of study, this deviation was exceeded in five of twelve instances. Since in this period there is most rapid change in concentration as equilibrium throughout the body is being approached, there is greater opportunity for error in the study.

TABLE I. URINE /BLOOD RATIOS AT HOURLY INTERVALS

SUBJECT	1	2	3	4	5	6	7	24	DIAGNOSIS
					Normal (Oral)				
E. L.			0.97	0.98	0.97	1.02		1.04	—
W. H.	0.76	0.88	0.93	0.99	0.95	1.02	0.99	1.02	—
W. F.	0.96	1.05	1.03	1.04	1.00	0.97			—
W. V.*	1.01	1.02	1.01	1.01	1.04	1.03	1.01	0.98	—
					Normal (i.v.)				
W. F.†	1.03	1.03	1.03	1.02	1.04	1.02	1.02	1.03	—
W. H.	1.07	1.02	1.00	0.98	0.98	0.98	0.94	0.99	—
W. V.	1.08	0.97	1.00	1.01	1.00	1.02		1.04	—
					Edematous (i.v.)				
R. S.			0.97	0.98	1.01	1.02		0.98	Arteriolo-nephrosclerosis
E. G.			1.05	1.06	1.00	1.04			ACVD
I. S.			1.06	1.04	0.99	0.98			ACVD
H. L.						1.00			ACVD
G. G.					0.99	1.02			RHD
					Edema Free (i.v.)				
R. S.			0.97	0.98	0.97			1.00	Arteriolo-nephrosclerosis
J. W.	1.04	1.06							ACVD
H. L.		0.98	1.00						ACVD
E. G.	0.99	0.98							ACVD
Avg. Ratio	0.98	0.99	1.01	1.01	0.99	1.01	0.99	1.01	
No. of determinations	6	6	13	14	13	12	4	8	

Mean U/B ratio for the 76 instances—1.00

*Urine milliliters per hour, 460 c.c.; arteriosclerotic cardiovascular disease.
†Urine milliliters per hour, 70 c.c.; rheumatic heart disease.

The average urine/blood ratio for the total of seventy-six instances was 1.00. This would indicate that the kidney in its function does not distinguish between water and its isotope heavy water. It also indicates that total body water determinations may be based upon analysis of urine as well as upon blood. In three normal subjects the heavy water was given orally and then intravenously on a subsequent occasion. The total body water expressed as per cent body weight varied no more than 2 per cent in each of these three subjects. In a fourth subject the difference was 6.4 per cent. The greater volume was obtained after oral administration, at which time the urine flow was held at approximately 500 ml. per hour, and correction for this large urine loss was not made.

SUMMARY

The average urine/blood ratio of deuterium oxide as determined seventy-six times on ten subjects was 1.00, indicating that the kidney in its function does not differentiate between water and deuterium oxide.

Determination of total body water may be based upon analysis of urine for deuterium oxide as well as of blood.

In suitable subjects deuterium oxide may be given by mouth for the determination of total body water.

The constant temperature water bath and temperature control mechanism used in these studies were built and furnished by the Holter Research Foundation, Helena, Mont.

Preparation of the heavy water for intravenous administration was done by the Eli Lilly Company.

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THE SERUM MUCOPROTEINS AS AN AID IN THE DIFFERENTIATION OF NEOPLASTIC FROM PRIMARY PARENCHYMATOUS LIVER DISEASE

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THE mucoprotein of serum is a glycoprotein complex characterized[1] by solubility in acid protein precipitants, viz., trichloracetic, sulfosalicylic and perchloric acids, as well as stability to heat, and a low isoelectric point[2] (pH 2.3). Serum mucoprotein may be conveniently isolated devoid of albumin and other globulins by differential precipitation[1, 3] employing perchloric and phosphotungstic acids. Indirect electrophoretic evidence[2, 4, 5] has indicated that the isolated mucoprotein resembles the alpha-1-globulin component in serum. The protein content (M) of mucoprotein in the normal adult varies from 55 per cent to 70 per cent; the nonglucosamine polysaccharide content, from 10 per cent to 18 per cent. The complete structure has not been established,[1] although aminosugars, lipids, etc., have been identified. An estimate of the mucoprotein content of serum may be conveniently obtained by measurement[1, 3, 6] of the preponderant protein (M) or the polysaccharide component (P_m).[7, 8]

The increased serum mucoprotein content has been reported to occur in a variety of infectious[1] or neoplastic[6, 9] diseases. The latter have not been associated with any abnormality in the polysaccharide/protein (P_m/M) ratio. An investigation of the influence of parenchymatous liver disease on the serum mucoprotein was instituted, when exceptions to these findings were noted[9] in a patient with advanced carcinoma of the prostate after the appearance of a homologous serum hepatitis, as well as in a number of patients with multiple myeloma. A reduced serum content of mucoprotein (M) associated with an increased proportion of polysaccharide to protein (P_m/M) was found[9, 10] among a number of patients with either uncomplicated infectious hepatitis, homologous serum hepatitis, or portal (Laennec's) cirrhosis. In contrast, the serum of patients with neoplastic infiltration of the liver, associated with hepatomegaly, showed elevated mucoprotein levels (M) with essentially normal polysaccharide/protein ratios (P_m/M).

These observations were deemed to be clinically useful in the differentiation of neoplastic from primary parenchymatous disease of the liver, and the results are presented at this time.

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METHOD

The normal sera were obtained from the members of the U. S. Public Health Service Hospital staff in apparent good health. The abnormal sera were obtained from patients hospitalized at the Clinical Research Unit of the National Cancer Institute, the Medical Service, U. S. Public Health Service Hospital, and the Medical Service, The Sinai Hospital, Baltimore, Md. In each case of neoplasia the diagnosis was established by tissue biopsy and/or autopsy examination. The diagnosis of acute hepatitis or portal cirrhosis was based upon the clinical and laboratory criteria generally employed. The diagnosis was confirmed by liver aspiration or autopsy examination in approximately one-third of the patients in this group. Concurrent complications unassociated with the primary hepatic disease are presented in Table I.

The procedure of Simkin[3] was used for the precipitation of mucoprotein. The protein component (M) of the precipitate was measured by a Biuret method;[11] the polysaccharide component (P_m), by a modification[9] of Shetlar's method.[8] The sera were obtained in the fasting state and were stored at −5° C. until examined.

In addition to the usual hematologic, renal, and chemical studies, the serum bilirubin,[12] albumin, globulin, and albumin/globulin ratio,[13] thymol turbidity,[14] zinc sulfate turbidity,[15] alkaline phosphatase,[16] bromsulfalein retention,[17] and plasma prothrombin time[18] were determined.*

RESULTS

The significant data are summarized in Table I. A somewhat lower mean content of M in the serum of normal adult women (54.3 mg. per 100 ml.), as compared to normal adult men (60.3 mg. per 100 ml.) was observed. Although the difference between the means is small, the individual M values appeared to be consistently lower among the women than among the men. It is believed that these differences would be statistically significant if a large enough group had been available for study. More than 95 per cent of the M values among the eighty-eight normal subjects fell within the range of 45 to 70 mg. per 100 ml. serum,† while 95 per cent of the estimations of the polysaccharide content (P_m) of serum mucoprotein in these normal subjects ranged from 5.7 to 13.2 mg. per 100 ml. The 95 per cent limits of the P_m/M ratios occurred between 0.132 and 0.196. Values beyond these limits were assumed to exceed the normal range.

Acute Hepatitis.—The observations on thirty-six patients with the diagnosis of acute infectious (viral) and homologous serum hepatitis are graphically represented in Figs. 1, 2, and 3, and summarized in Table I. A mean value for the mucoprotein (M) of 38.6 mg. per 100 ml. was obtained, as compared to a mean of 57.6 mg. per 100 ml. with eighty-eight normal subjects. The initial sera from the patients with hepatitis were obtained in most instances within several days after admission to the hospital. The serum mucoprotein (M) content was below the normal, or in the low normal range in twenty-seven of the thirty initial sera from patients with infectious hepatitis (Fig. 1). M levels in the normal range occurred in two patients in whom clinical and laboratory data were more consistent with the "cholangiolitic" type of hepatitis.[19] A

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†The range among the men was 48 to 74 mg. per 100 ml., and among the women was 40 to 70 mg. per 100ml. If the data to be presented were expressed according to sex, certain of the differences to be reported among the abnormal groups would be more clearly defined. The preponderance of the patients available for this study were men.

TABLE I. THE SERUM MUCOPROTEINS IN PATIENTS WITH HEPATOMEGALY OR JAUNDICE

ESTIMATION		NORMAL SUBJECTS			ACUTE INFECTIOUS OR HEMOLOGOUS SERUM HEPATITIS	PORTAL CIRRHOSIS (UNCOMPLICATED)*	HEPATIC METASTASES	OBSTRUCTIVE OR INFLAMMATORY BILIARY DISEASE
		MALE	FEMALE	TOTAL				
Mucoprotein (M) as Biuret Peptide Mg. per cent	No. Patients	46	43	89	36	34	25	31
	Mean	60.3	54.3	57.6	38.6	32.5	116.1	82.9
	Median	59.8	54.8	56.7	38.3	32.1	115.3	75.3
	Range	48.8-74.2	39.1-70.6	39.1-74.2	12.4-77.4	13.9-65.1	44.7-177.0	41.4-191.7
	S. D.	7.4	7.7	7.5	12.3	11.8	31.9	30.9
	S. E.	1.1	1.2	0.8	2.1	2.0	6.8	5.7
Polysaccharide (P_m) of Mucoprotein as Galactose-Mannose Mg. per cent	No. Patients	45	43	88	34	31	25	31
	Mean	9.5	9.3	9.4	8.4	7.6	21.0	15.7
	Median	9.8	9.1	9.4	8.2	7.4	19.4	13.9
	Range	4.9-13.6	5.8-13.2	4.9-13.6	3.9-13.2	3.9-12.5	10.0-45.7	10.0-36.4
	S. D.	2.6	2.0	2.3	2.3	2.2	6.3	5.5
	S. E.	0.4	0.3	0.2	0.4	0.4	1.3	1.1
(P_m/M)	No. Patients	45	43	88	34	31	25	31
	Mean	0.16	0.17	0.17	0.24	0.25	0.19	0.19
	Median	0.16	0.17	0.17	0.22	0.25	0.19	0.19
	Range	0.09-0.22	0.12-0.22	0.09-0.22	0.14-0.47	0.15-0.35	0.15-0.25	0.13-0.30
	S. D.	0.022	0.026	0.023	0.057	0.055	0.024	0.058
	S. E.	0.003	0.004	0.002	0.01	0.01	0.005	0.006

*Excluded from the statistical summary of this category are six cases of portal cirrhosis with obvious extrahepatic complications, noted in the text. Included in this category are three patients who had a clinically unrecognized neoplasm at the time the sera were obtained. The lesions were malignant rectal polyp, 1; early esophageal carcinoma, 1; malignant hepatoma, 1. No evidence of metastases were present.

progressive fall in M levels to the subnormal range was observed upon serial study of several patients in which the initial values were in the low normal range. A clinical diagnosis of infectious hepatitis was made in only a single patient with an elevated M content (77 mg. per 100 ml.). He had had several previous episodes of jaundice of undetermined etiology. No liver biopsy was obtained. A reduced serum mucoprotein (M) content was observed in all six patients with homologous serum hepatitis.

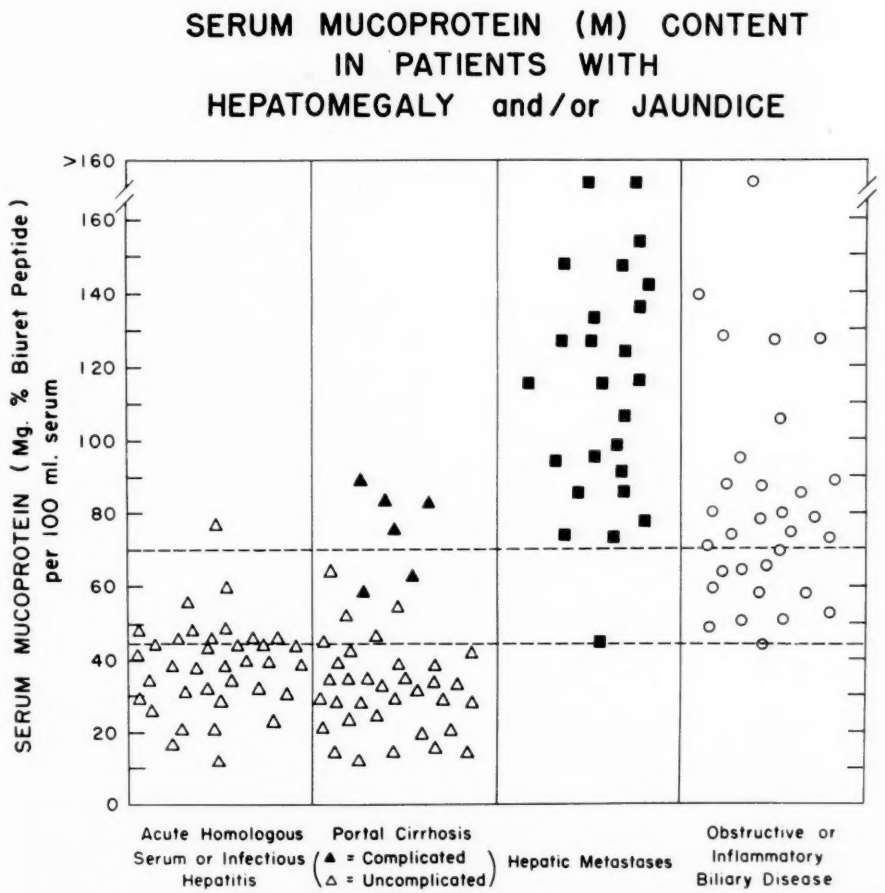


Fig. 1.—The serum mucoprotein (M) content, measured as Biuret-peptide (casein as standard), among patients with hepatomegaly and/or jaundice due to common etiological factors designated at the base of the chart. The limit of the normal range (dashed horizontal lines), is approximated without regard to a possible sex difference (see text footnote). The cases of complicated portal cirrhosis, indicated with a black triangle, have been described in the text.

The polysaccharide (P_m) content of the mucoprotein in the hepatitis group remained essentially in the normal range (Fig. 2). The P_m/M ratio was elevated (0.20 or greater) in twenty-seven of thirty-four sera in which this determination was performed (Fig. 3). This change in the composition of mucoprotein was principally the result of a reduced proportion of protein (Biuret peptide) rather than of a rise in the total amount of polysaccharide.

Four patients with carbon tetrachloride poisoning and liver damage of varying degree were studied. The mucoprotein (M) content of the initial sera was below the normal range in two of these patients. The remaining two cases showed M levels above the normal range. Serial determinations were possible in one patient shortly after the simultaneous acute onset of severe hepatic and renal damage. The changes in M content observed during the course of this

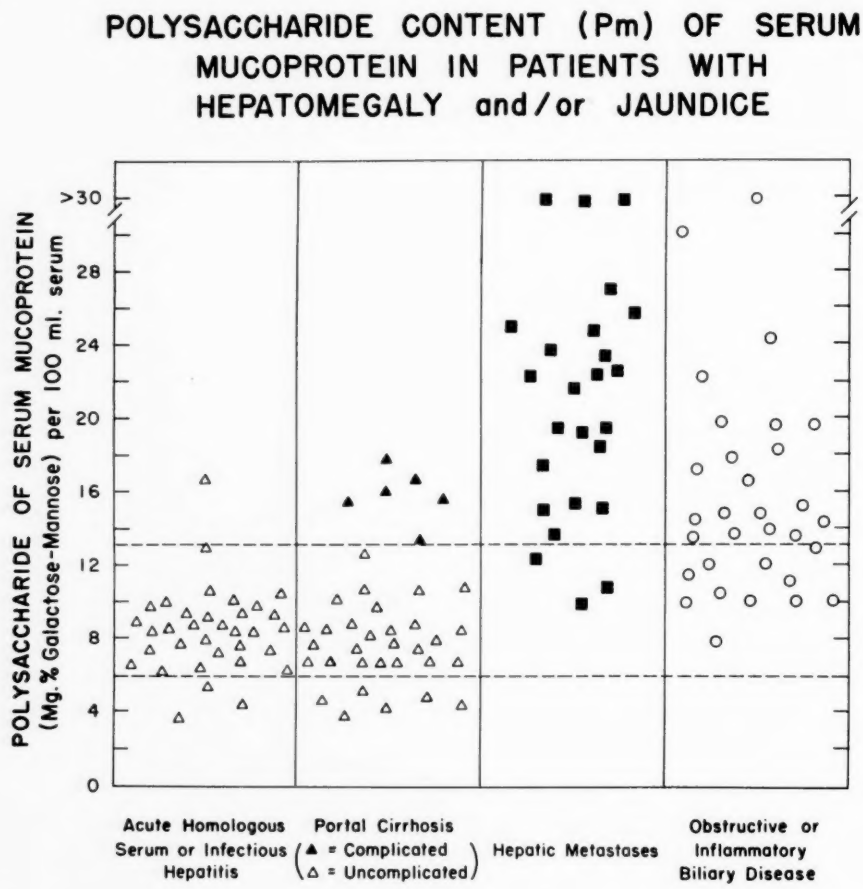


Fig. 2.—The polysaccharide (P_m) content of serum mucoprotein among patients with hepatomegaly and/or jaundice. Symbols and groups as in Fig. 1.

patient's disease were so striking as to suggest that the M level was a resultant of simultaneously-acting intrahepatic and extrahepatic factors. Fig. 4 illustrates the sudden rise from reduced mucoprotein levels to markedly elevated levels which occurred in this patient a few days after admission. This marked increase in the M content developed when the patient first began to show evidence of improved hepatic function, but while renal damage, hypertension, pulmonary congestion, and heart failure were still progressive. One of the two patients with high mucoprotein levels was in a moribund state from renal insufficiency

and died within several hours after serum was obtained for examination. The other showed signs of severe gastrointestinal and renal damage, with relatively slight evidence of hepatic injury. Such elevations have been previously noted[9] in infectious hepatitis when extrahepatic necrosis or infection supervened.

The M content was either in the normal or elevated range and the ratio, P_m/M, was normal among all seven patients with hepatomegaly and fatty infiltration of the liver, diagnosed by aspiration biopsy.

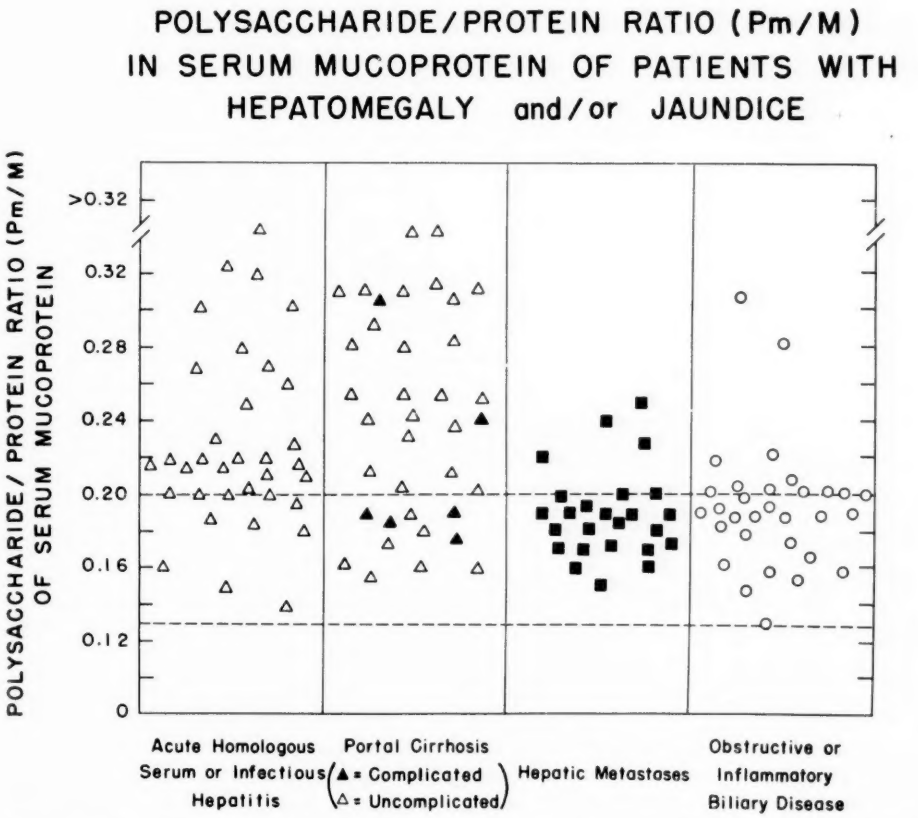


Fig. 3.—The polysaccharide/protein ratio (P_m/M) in the serum mucoprotein among the patients represented in the preceding figures.

The influence of hepatitis associated with infectious mononucleosis on the serum mucoprotein level was observed in three patients. Increased M levels and normal P_m/M ratios were present in all three patients, in contrast to the observations in the primary infectious or serum hepatitis group.

Serial determinations of the M content from representative patients with infectious or homologous serum hepatitis are presented in Fig. 5.

Portal Cirrhosis.—The mucoprotein (M) levels in forty patients with portal cirrhosis were reduced in thirty, normal in six, and increased in four. A mean serum mucoprotein value of 32.5 mg. per 100 ml. (normal = 57.6 mg. per

100 ml.) was obtained from thirty-four patients with apparently uncomplicated portal cirrhosis. The polysaccharide (P_m) content of the serum mucoprotein remained essentially unchanged (Fig. 2), resulting in an elevated P_m/M ratio in twenty-six of thirty-seven sera (Fig. 3). Each of the four cases of portal cirrhosis with increased M levels manifested obvious extrahepatic complications, viz., lobar pneumonia, 2; adenocarcinoma of the kidney with metastases, 1; and gastric adenocarcinoma with regional and hepatic metastases (in a cirrhotic liver), 1. Extrahepatic complications were also present in two of the four cases with M levels in the normal range. These were *B. proteus* septicemia and acute pyelonephritis, respectively. The mucoprotein (M) concentration was normal in only three of thirty-four patients with apparently uncomplicated portal cirrhosis (Fig. 1).

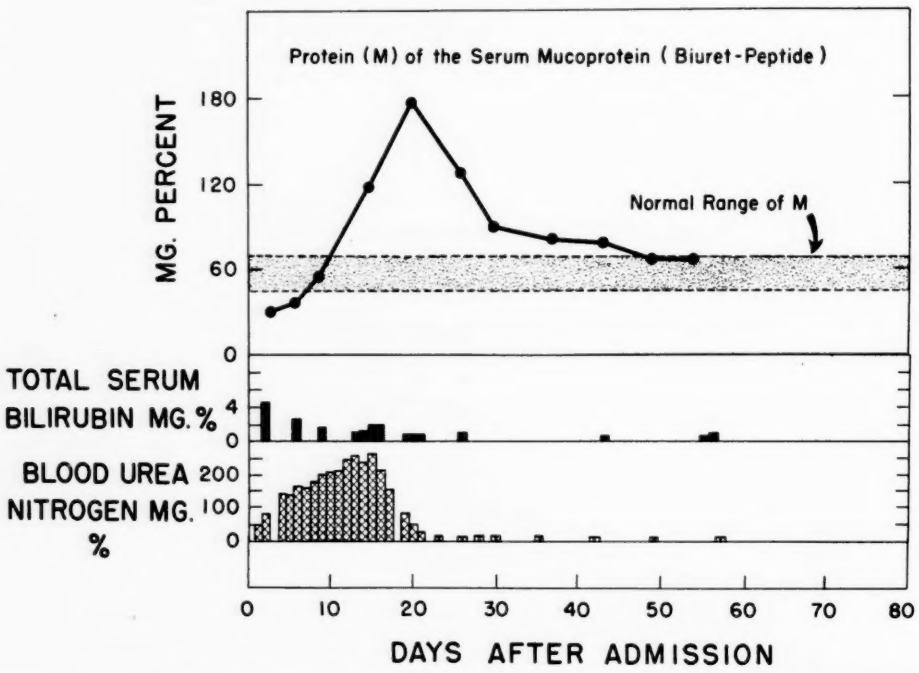


Fig. 4.—Fluctuations in the serum mucoprotein (M) level in a patient with the simultaneous onset of severe hepatic and renal damage due to carbon tetrachloride poisoning. Recovery of hepatic function preceded the improvement in signs of renal insufficiency and associated systemic manifestations therewith.

Hepatomegaly with Hepatic Metastases.—An increased serum mean mucoprotein content (115.3 mg. per 100 ml.) was observed in twenty-five patients with hepatomegaly secondary to neoplastic involvement of the liver. The M level in this group was conspicuously different from the hepatitis and cirrhosis groups, in which the means were 38.6 and 32.5, respectively (Table I, Fig. 1). Elevated M levels were observed among twenty-four of the twenty-five patients. The single instance of a normal M level was found in a patient with a massively en-

larged liver who had had an enucleation of the eye because of choroid (retinal) melanoma. At postmortem examination metastases were exclusively confined to the liver. The liver weighed 8,250 Gm., and only a few intact liver cords were observed. Clinical signs of severe hepatic insufficiency, probably on a hepatic-replacement basis, were evident in this patient.

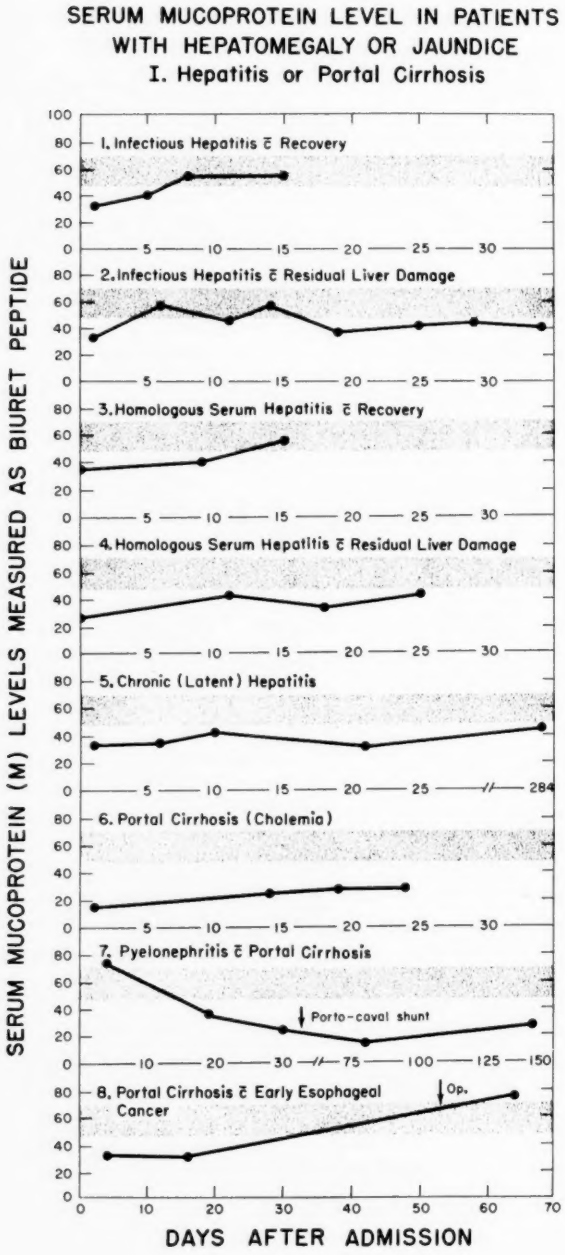
Among the hepatic metastases group the polysaccharide content of serum mucoprotein (Fig. 2) was increased in proportion to the increase in the protein, so that no significant changes occurred in P_m/M ratios (Fig. 3).

Obstructive and Inflammatory Biliary Disease.—The mean mucoprotein content of patients with obstructive or inflammatory biliary disease (82.9 mg. per 100 ml.) was only slightly above the upper limit of the normal, but was significantly (Table I) above the means of the hepatitis and cirrhosis groups. The M concentration was elevated in twenty and not below the lower limits of normal in any of thirty-one patients with either acute or chronic cholecystitis with cholelithiasis, cholangitis, common duct stone, carcinoma of head of pancreas, or carcinoma of gall bladder. The P_m/M ratios (Fig. 3), although variable, were not significantly elevated. The mucoprotein value was elevated in all (ten) patients with acute cholecystitis. Normal M concentrations were observed in four patients with acute biliary obstruction from common duct stone, in three patients with chronic cholecystitis and cholelithiasis, in three of eight patients with obstruction from carcinoma of the head of the pancreas, and in one patient with carcinoma confined to and arising in the gall bladder. Serial determinations of the M level from representative cases of hepatitis, cirrhosis, hepatic metastases, and obstructive or inflammatory biliary disease are presented in Fig. 5.

Hepatomegaly with Congestive Heart Failure.—An elevated M level with normal P_m/M ratios was found in eleven of twelve patients with a hepatomegaly secondary to acute or chronic congestive heart failure. Two patients with "cardiac cirrhosis" showed reduced M levels and high P_m/M ratios similar to those observed in portal cirrhosis.

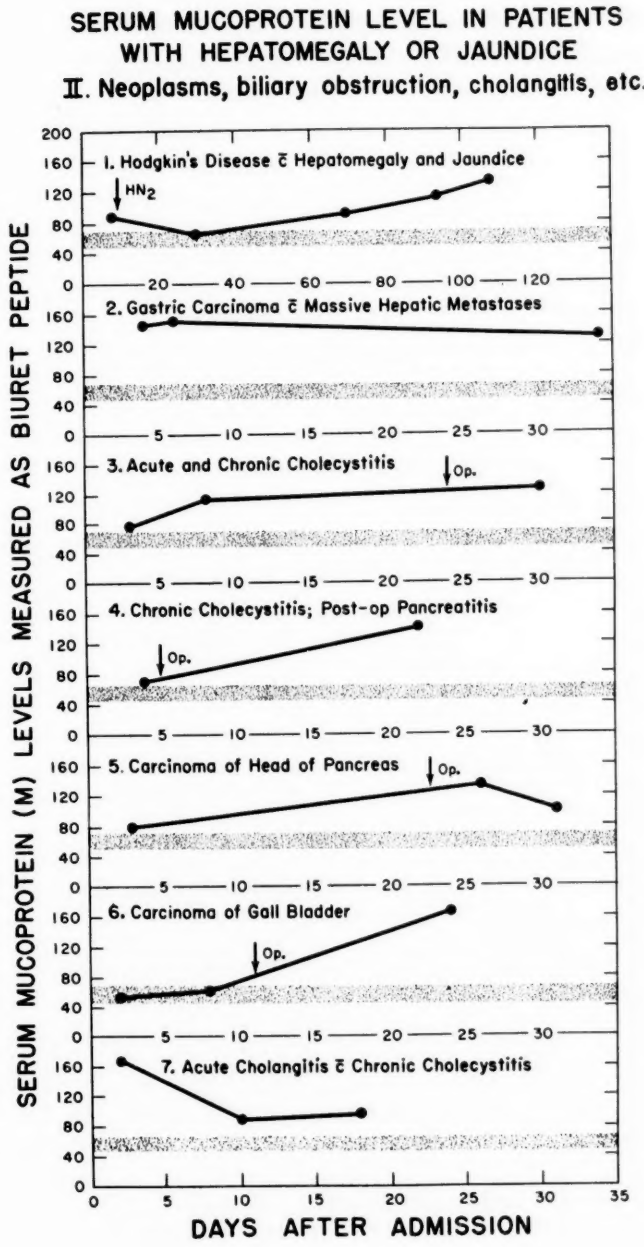
Miscellaneous.—Mucoprotein determinations on patients with hepatomegaly due to a variety of other pathologic conditions were also performed. An elevated mucoprotein (M) content with normal P_m/M ratios was found in the following instances: sickle-cell anemia, 1; scleroderma, 1. Normal M levels with essentially normal P_m/M ratios were observed in the following: salmonella sepsis with jaundice, 1; osteosclerotic anemia, 1; diabetes mellitus, 2. Reduced mucoprotein levels and high P_m/M ratios were observed as follows: generalized sarcoidosis, 2; diabetes mellitus, 2; kala-azar, 1; toxic nodular goiter (Plummer's type) with hepatomegaly and cardiac decompensation, 1.

Comparative Liver Function Tests.—The changes in the mucoprotein level in the various disease states were independent of the abnormality revealed by the serum bilirubin, thymol turbidity, zinc sulfate, alkaline phosphatase, prothrombin time, or bromsulfalein tests.


SERUM MUCOPROTEIN LEVEL IN PATIENTS
WITH HEPATOMEGALY OR JAUNDICE
I. Hepatitis or Portal Cirrhosis
SERUM MUCOPROTEIN (M) LEVELS MEASURED AS BIURET PEPTIDE
1. Infectious Hepatitis ⊂ Recovery
2. Infectious Hepatitis ⊂ Residual Liver Damage
3. Homologous Serum Hepatitis ⊂ Recovery
4. Homologous Serum Hepatitis ⊂ Residual Liver Damage
5. Chronic (Latent) Hepatitis
6. Portal Cirrhosis (Cholemia)
7. Pyelonephritis ⊂ Portal Cirrhosis
Porto-caval shunt
8. Portal Cirrhosis ⊂ Early Esophageal Cancer
Op.
DAYS AFTER ADMISSION

A.

Fig. 5A.—Serial determinations of the mucoprotein (M) level in representative patients with hepatitis, cirrhosis, hepatic metastases, and obstructive or inflammatory biliary disease. The normal range of M levels in the serum is indicated by the shaded areas. The M level is expressed as milligram per cent Biuret-peptide. The X axis varies according to the duration of hospitalization.


SERUM MUCOPROTEIN LEVEL IN PATIENTS
WITH HEPATOMEGALY OR JAUNDICE
II. Neoplasms, biliary obstruction, cholangitis, etc.
SERUM MUCOPROTEIN (M) LEVELS MEASURED AS BIURET PEPTIDE
1. Hodgkin's Disease c̄ Hepatomegaly and Jaundice
HN2
2. Gastric Carcinoma c̄ Massive Hepatic Metastases
3. Acute and Chronic Cholecystitis
Op.
4. Chronic Cholecystitis; Post-op Pancreatitis
Op.
5. Carcinoma of Head of Pancreas
Op.
6. Carcinoma of Gall Bladder
Op.
7. Acute Cholangitis c̄ Chronic Cholecystitis
DAYS AFTER ADMISSION

B.

Fig. 5*B*.—Serial determinations of the mucoprotein (M) level in representative patients with hepatitis, cirrhosis, hepatic metastases, and obstructive or inflammatory biliary disease. The normal range of M levels in the serum is indicated by the shaded areas. The M level is expressed as milligram per cent Biuret-peptide. The X axis varies according to the duration of hospitalization.

DISCUSSION

The mean levels of serum mucoprotein (M) in the principal groups studied were: acute hepatitis, 38.6 mg. per 100 ml.; uncomplicated portal cirrhosis, 32.5 mg. per 100 ml.; hepatomegaly with hepatic metastases, 116.1 mg. per 100 ml.; obstructive or inflammatory biliary disease, 82.9 mg. per 100 ml.; normal subjects, 57.6 mg. per 100 ml. Reduction in the serum mucoprotein (M) level was found in 85 per cent of patients with acute infectious or homologous serum hepatitis and 87 per cent of patients with uncomplicated portal cirrhosis. The possible diagnostic significance of these observations is enhanced by the high incidence of elevated mucoprotein (M) levels in the other common pathologic conditions associated with hepatomegaly or jaundice. An elevated mucoprotein (M) level was observed in approximately 95 per cent of the patients with clinically-evident enlargement of the liver due to neoplastic infiltration. A low M level, as well as an elevated P_m/M ratio, consequently appears to represent an aid in the diagnosis of hepatomegaly and jaundice when attempting to differentiate primary parenchymatous insufficiency from neoplastic involvement of the liver.

The M level may also provide some aid in the differentiation of hepatitis or cirrhosis from obstructive or inflammatory biliary conditions warranting surgical intervention. The latter disease states are usually associated with elevated levels, or, less frequently, with levels in the normal range. The data indicate that reduced M levels would seldom occur in patients with cholecystitis, cholelithiasis, common duct stone, cholangitis, carcinoma of head of pancreas, or carcinoma of gall bladder. Although a larger experience with surgical biliary disease may reveal a few exceptions to these findings, the present observations suggest that a diagnosis of obstructive biliary disease, or metastatic neoplasia should be made with caution in the presence of a reduced serum mucoprotein content and an increased P_m/M ratio.

The concentration and composition of mucoprotein in the serum appears to represent the resultant of both intrahepatic and extrahepatic processes. The intrahepatic component becomes evident with the development of diffuse parenchymatous hepatic degeneration. The extrahepatic elements, which tend to increase the serum content of mucoprotein, include infection, neoplasia, shock,[20] and surgical procedures.[21] The common factors in these conditions, viz., cellular proliferation, inflammation, exudation, hemorrhage, or necrosis, have been shown experimentally to increase the level of mucoprotein-like substances in the serum.[20] It is probable that a release of excessive amounts of mucoprotein into the blood stream occurs from sites of major tissue alteration. It has been observed[9] that, in the presence of significant extrahepatic complications, the mucoprotein content of the serum may be normal or elevated, and obscure the presence of diffuse parenchymatous hepatic insufficiency.

A probable relationship of serum mucoprotein to the alpha-1-globulins has been demonstrated by isolation experiments.[2, 4, 5] The increased serum mucoprotein level in patients with advanced cancer is consistent with the increase in alpha-globulin concentration reported in electrophoretic studies by Mider and associates[22] on cases of "late cancer." No consistent changes in the alpha-

globulin content of the serum as measured electrophoretically has been reported[23, 24] in patients with hepatitis or cirrhosis. Technical factors involved in electrophoresis complicate the detection of changes in the various individual components of the alpha-globulins. The reduction and alteration of serum mucoprotein in the presence of uncomplicated hepatic insufficiency are not the only findings which suggest that heretofore unrecognized changes in the alpha-globulins probably develop during episodes of hepatitis. Since the initiation of our studies, Havens[25] reported the absence of an alpha-globulin component, i.e., polysaccharide C-reactive protein, in acute infectious hepatitis. Although no studies were performed on patients with cirrhosis, an attempt was made to relate this observation to the pathogenesis of viral infection. Reduction in serum level of cholinesterase, also a component of the alpha-globulins, has been noted[26] in the presence of acute or chronic hepatic insufficiency. The determinations of serum mucoprotein would seem to provide a crude index of alteration in alpha-globulins, analogous to the numerous serum tests correlated with the status of the serum beta or gamma globulins.

A precise evaluation of the diagnostic significance of the serum mucoprotein must await further study on large numbers of patients. For the present, it may be assumed that reduced serum mucoprotein levels will be observed in most patients with hepatitis or cirrhosis. A mucoprotein estimation may provide a useful aid in distinguishing primary parenchymatous disease of the liver from neoplastic infiltration or obstructive biliary disease.

SUMMARY

Among eighty-eight normal adult subjects, the mean content of mucoprotein (M) was 57.6 mg. protein (Biuret peptide) per 100 ml. serum; the mean polysaccharide (P_m) content (measured as galactose-mannose) was 9.4 mg. per 100 ml.; and the mean P_m/M ratio was 0.17. More than 95 per cent of the M values were found between 45 and 70 mg. per 100 ml. The mean and lower limits of normal in women were somewhat lower than in men.

In the various categories of hepatomegaly and jaundice studied, the mean M levels were: acute hepatitis, 38.6 mg. per 100 ml.; portal cirrhosis (uncomplicated), 32.5 mg. per 100 ml.; hepatomegaly with hepatic metastases, 116.1 mg. per 100 ml.; obstructive or inflammatory biliary disease, 82.9 mg. per 100 ml. The P_m/M ratios were increased in patients with hepatitis or cirrhosis, but not significantly altered in the hepatic metastases and biliary disease groups. A reduced mucoprotein (M) content of the serum was observed in thirty-two of thirty-six cases of acute homologous or infectious hepatitis, and in thirty-two of forty patients with portal cirrhosis, but in no patient with hepatic metastases or obstructive or inflammatory biliary disease.

An impairment in the alpha-globulin formation is probably related to the reduced content of mucoprotein in patients with diffuse liver disease.

The potential diagnostic aid of serum mucoprotein estimations in the differentiation of disease states characterized by hepatomegaly and jaundice is discussed.

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THE EFFECTS OF MODIFIED HUMAN GLOBIN ON RENAL FUNCTION IN CIRRHOSIS OF THE LIVER

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PLASMA protein deficit, excess of antidiuretic pituitary hormone, or of sodium-retaining corticoid substances, and reduced renal blood flow are factors in the formation of ascites in patients with cirrhosis.[1-5] The use of salt-poor albumin as a therapeutic tool in the treatment of cirrhosis held promise, but has enjoyed only moderate success because of cost and the necessity for long continued therapy.

When modified human globin* became available, it was decided to study the effects of this substance on cirrhotics in regard to renal function and sodium excretion as compared with normal subjects.[6]

METHODS

A group of twelve subjects were studied. The diagnosis of cirrhosis of the liver was established by history, physical examination, laboratory tests, and in most cases a liver biopsy. Six of the subjects had ascites and peripheral edema; six had none. All patients were on a hospital low sodium, high protein, low fat diet with added vitamins. Those who had been on mercurial diuretics were taken off the drug for at least forty-eight hours before the experiment. Patients were studied in the fasting state at least ten hours after their last meal. They were hydrated with about 1,000 ml. of water before the experiments to assure adequate urine flows. Renal clearances of inulin and PAH were determined, as well as minute excretions of sodium. Not all procedures were carried out in all patients. The constant infusion technique for renal clearances was used. Clearance periods averaged from five to fifteen minutes, depending upon urine flow. An indwelling catheter assured accurate urine collections by bladder washout and air injection and evacuation. Determinations of inulin and PAH in plasma and urine were made by the usual methods.[7, 8] Blanks of urine and blood were collected prior to the test. Sodium concentrations in urine were determined with the flame photometer using lithium as the internal standard. Two or three control periods using 5 per cent glucose in water were collected prior to the administration of globin. Infusion rates during control periods and during globin administration were kept constant. In ten cases no more than 32 Gm. of globin were administered in more than one hundred and forty minutes, in some much less; in two cases (C. C., and H. D.) globin was infused at a rapid rate, so that at least 32 gm. were administered in sixty minutes. Hematocrits were done during control periods and after globin on heparinized blood in Wintrobe tubes centrifuged for thirty minutes at 2,500 r.p.m./min.

RESULTS

I. *Effect of Globin Administration on Renal Function in Cirrhotics With Ascites (A, Table I).*—In these six patients, all showed a slight but definite

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*The modified human globin was supplied through the courtesy of Sharp & Dohme, Glenolden, Pa. The material is supplied in bottles containing 16 Gm. (one unit) of the lyophilized material with approximately 1.0 Gm. N_aCl. The globin is dissolved in 400 ml. of 3.5 per cent glucose in water, and is then isotonic. The colloidal osmotic pressure is equivalent to approximately 400 c.c. of 8 per cent albumin.

TABLE I. RENAL DATA ON PATIENTS GIVEN GLOBIN

NAME, AGE, SEX GM. GLOBIN/TOTAL MIN.	PERIODS	URINE FLOW (C.C./MIN.)	C_{PAH} (R.P.F.)	C_{IN} (G.F.R.)	FILT. FRACT.	NA EXCRETION MM/MIN. X10^{-3}
		A. Patients With Ascites				
R. C., 54, M.	Control (3)	3.7	509			9.1
22 /70	Globin (3)	8.4	547			29.7
	Globin (3)	9.5	511			34.5
W. C. (I), 68, M.	Control (3)	2.6	103			
22 /43	Globin (3)	1.9	104			
	Globin (3)	2.8	522			
W. C. (II), 68, M.	Control (3)	2.0	193			63.5
30 /80	Globin (3)	2.6	302			85.5
	Globin (3)	2.5	229			109.1
	Globin (2)	3.0	230			120.5
C. K., 46, F.	Control (2)	4.0	558			1.7
32 /82	Globin (4)	6.7	463			2.3
	Globin (4)	8.8	542			5.4
M. B., 44, F.	Control (3)	0.27	72			3.2
32 /140	Globin (3)	0.2	106			1.7
	Globin (4)	0.5	308			6.8
	Globin (4)	1.7	398			63.0
H. D., 51, M.	Control (3)	11.6	693	142.4	0.203	28.7
48 /70	Globin (2)	14.4	701	125.8	0.179	56.1
	Globin (2)	13.4	648.5	117.9	0.182	110.6
	Globin (2)	13.6	699.5	108.4	0.155	129.2
		B. Patients Without Ascites				
J. H., 52, M.	Control (3)	8.8	379	71.0	0.187	
3 /80	Globin (3)	5.3	325	103.0	0.317	
	Globin (3)	1.7	331	69.0	0.208	
D. R., 53, M.	Control (3)	0.7	218			29.0
25 /90	Globin (3)	1.0	225			52.0
	Globin (4)	2.6	261			98.0
L. Q., 43 F.	Control (3)	6.7	491	121	0.246	15.5
32 /110	Globin (3)	6.8	399	117	0.293	45.1
	Globin (3)	6.6	432	119	0.275	78.1
J. R., 43, M.	Control (3)	6.3	560	115	0.205	24.0
32 /100	Globin (3)	9.7	545	148	0.271	35.8
	Globin (3)	5.7	452	143	0.316	38.4
M. S., 44, F.	Control (3)	1.9	313	67	0.214	67.1
32 /100	Globin (3)	4.8	316	59	0.187	176.0
	Globin (4)	6.1	316	65	0.206	230.4
C. C., 44, M.	Control (2)	9.1	372	62.2	0.203	19.9
32 /60	Globin (3)	9.4	467	76.2	0.163	302.2
	Globin (2)	8.3	484	67.5	0.139	333.1

All data represent the average of the number of consecutive periods in parentheses. All data converted to standard 1.73 m^2.

increase in urine flow per minute. This immediate effect of modified human globin is more marked than is seen with the administration of an equivalent weight of serum albumin. In three of these six patients there was an apparent significant increase in the renal plasma flow over control levels. Patient W. C. was tested on two occasions, both times with ascites. In the first experiment (W. C. I) the patient had a rapidly reaccumulating ascites with marked peripheral scrotal and penile edema, and on the second occasion (W. C. II) after approximately 5 liters of ascitic fluid had been removed by paracentesis. It will be noted that the effects of similar doses of globin caused a greater increase in urine flow on the second occasion with a diminution of the amount of ascites whereas the reverse effects occurred as regards the renal plasma flow.

In Subject H. D. globin was infused at a fairly rapid rate (17 ml. per minute) and seemed to induce no remarkable changes in renal plasma flow; there was a tendency to a gradual fall in glomerular filtration rate and filtration fraction.

II. *Effects of Globin Administration on Renal Function in Cirrhotics Without Ascites (B, Table I).*—In this group of patients (excluding subject C. C.) none showed any remarkable changes in urine flow or renal plasma flow over control levels. The glomerular filtration rates in four patients of this group likewise showed no remarkable variations from the control levels, although two showed some increase (J. H., a transient rise, and J. R., a sustained rise).

In subject C. C. globin was infused fairly rapidly (13 ml. per minute) and induced a sustained increase in renal plasma flow with a transient rise in filtration rate and a tendency to a decrease in filtration fraction.

III. *Effect of Globin on Sodium Excretion (See Table I).*—The most significant and consistent change observed in the cirrhotics treated with globin was a rise in the rate of excretion of sodium. The rise in excretion varied from 58 per cent to over a 350 per cent increase in urinary excretion of sodium. While most patients had more than a threefold increase when globin was infused at a rate of approximately 0.3 Gm. per minute, at the usual infusion rate, in one subject (C. C.) without ascites, there was over a fifteen fold increase in sodium excretion per minute without a remarkable rise in urine flow, when globin was administered at a rate of 0.5 Gm. per minute.

IV. *Effects on Blood Pressure and Hematocrit.*—No significant alterations in the blood pressure or hematocrits were observed during the experimental period in most patients studied as acute short term experiments; two subjects who showed a falling hematocrit received globin at rapid rates.

DISCUSSION

Many investigators have found that the multitude of factors acting in the development of edema and ascites in cirrhosis are rarely controlled completely by any single therapeutic agent. Therapeusis of cirrhosis now hinges on agents which will alter one or more of the deleterious factors contributing to the clinical picture. Although modified human globin would probably help in refilling a depleted protein metabolic pool, and reduce or reverse the positive sodium balance usually found in cirrhotics, the results of prolonged administration are yet to be determined.

Although the studies carried out in these patients were of short duration, we regard the evidence of consistently increased sodium excretion as conclusive proof of a useful action. The acute renal effects of globin administration are in the right direction: a tendency to diuresis, increased renal plasma flow, and increased glomerular filtration rate. It has been firmly established that strict sodium restriction will retard the formation of ascites.[9] Two of the four patients without ascites had normal glomerular filtration rates; normal filtration rates have been previously reported in patients with ascites.[4] This would indicate a higher level of sodium reabsorption as the cause of the sodium retention regularly

observed, and a decline in this level following globin infusion. How the infusion acts on tubular reabsorption is unknown, but osmotic diuresis, as with mannitol, may contribute, since the globin is excreted in the glomerular filtrate.

Previous studies in normals[6] indicate that administration of modified human globin has no striking effects upon the excretion of salt and water. In the studies of Orloff, Welt, and Stowe[10] with serum albumin it was apparent that the primary diuretic action of albumin in edematous patients was to promote a diuresis of water, and if water is lost in excess of salt, to elevate the concentration of sodium in the extracellular compartment and an increased excretion of sodium ensues. In normals given serum albumin, response depends upon the balance of actions between the colloid osmotic pressure and the plasma volume.[11] Although modified human globin, as now available, is probably a mixture of various molecular sizes, approximately 90 per cent is one-half that of serum albumin. This fact would account for a considerably greater theoretical oncotic effect than similar amounts of serum albumin, and could account for a diuresis on the basis of an expanding plasma volume. This fact is borne out in two subjects who had a falling hematocrit with a rapid globin infusion. Since considerable quantities of globin may pass the glomerular barrier and are reabsorbed by the tubules, sodium reabsorption might be partly blocked even though PAH excretion is not interfered with.

Although the globin used in these experiments contained approximately 1 Gm. N_aCl per unit (17 meq Na) this amount of sodium was usually administered over a relatively long period of time and was followed by a more marked sodium diuresis than one would expect from equivalent concentrations noted in "salt loading" studies in cirrhotics.[12] As with serum albumin, modified human globin tends to increase filtration rate and renal plasma flow, with a fall in filtration fraction. It would seem that a slight hyperemia is induced in the glomeruli. The unusual feature of modified human globin as compared to the published data on the effects of albumin infusion on chloride and presumably on sodium excretion, is that with globin an electrolyte diuresis occurs without a remarkable water diuresis. The administration of globin did not cause detectable weight loss or significantly retard accumulation of ascitic fluid. The maximal rise in sodium excretion was 0.3 meq. per minute, or 18 meq. per hour for a few hours; the average rise was far less.

The present study did not evaluate such factors as portal venous pressure or ascites as causes in the alterations noted in renal hemodynamics and electrolyte excretions. The refractoriness which might be encountered in some patients may be related to these factors.

CONCLUSIONS

Single intravenous infusions of modified human globin were administered to twelve cirrhotics, six with ascites and six without. The effects of these infusions on renal dynamics, water and sodium excretion, glomerular filtration rate and renal plasma flow were noted.

There was a consistent and often marked rise in the excretion of sodium per minute during the administration of globin. This was not associated with weight loss or significant slowing in the subsequent reaccumulation of ascites.

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RATES OF EQUILIBRATION OF INULIN AND MANNITOL BETWEEN PLASMA AND INTERSTITIAL WATER IN EDEMATOUS STATES

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QUANTITATIVE measurement of the extracellular fluid phase is essential to an understanding of disturbances in body water distribution. Numerous solutes have been used to measure the volume of the extracellular phase. These include thiocyanate,[1-4] sodium,[5] sulfate,[2] bromide,[6, 7] and chloride.[7-9] Recently a series of three carbohydrates, sucrose,[2, 10] mannitol,[11-14] and inulin[10, 15-17] have been extensively studied. These substances obviate the disadvantage of variable entrance of electrolytes into cells, diffuse into a smaller space of distribution, and presumably more accurately estimate the extracellular volume.

Inulin spaces can be measured by the infusion-recovery method. The quantity of inulin recovered in the urine following a continuous intravenous infusion is divided by the plasma concentration at equilibration to obtain the volume of distribution of inulin. It has been reported that in normal subjects approximately five hours of continuous infusion are required to attain uniform distribution of inulin.[16, 17] It has been concluded[17] that the inulin space represents the best measure of extracellular volume thus far available, since the inulin space as measured by the infusion-recovery method yields the lowest values for extracellular fluid volume plus the fact that all previous substances used are known to either enter cells or to be partially metabolized. The disadvantages in the use of inulin are (1) the necessity for a continuous intravenous infusion, (2) relatively difficult analytic methods, and (3) the slow rate of diffusion of the large inulin molecule (molecular weight, 5,100) into interstitial fluids.

Recently, an infusion-decrement technique using mannitol has been reported for the measurement of the extracellular volume.[14] The volume and distribution of mannitol were calculated by dividing the total clearance by the decrement with time of the natural logarithm of the plasma mannitol concentration. The volumes of distribution of mannitol and inulin were measured simultaneously in normal human subjects, and close correspondence between the inulin and mannitol spaces was obtained.[18] The authors concluded that these data attested to the validity of both methods for measuring the extracellular fluid volume in normal man.

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Investigations employing mannitol and inulin have been for the most part limited to normal human subjects. It is desirable that methods proposed for the measurement of extracellular volume be applicable to edematous states. Since in the use of both inulin and mannitol, continuous infusion is maintained to the point of equilibration between plasma and interstitial water, it is essential to know how much time is required before equilibration occurs between plasma and edema fluid. Although a few reports are available on isolated patients in this connection,[13, 19] the problem has been insufficiently investigated and is the basis of the present study.

METHODS

In the present investigation, three groups of subjects were studied: (1) normal adult men, (2) cirrhotic patients with ascites, and (3) patients with congestive heart failure. The subjects were allowed food and water ad libitum, but were kept recumbent in bed throughout the experimental procedure. No preliminary hydration was employed. Prior to infusion, blood samples were obtained for determination of plasma blanks. A priming injection of mannitol and inulin was followed by a constant sustaining infusion for eight and twenty-four hours, respectively. Plasma levels of inulin were maintained between 20 and 40 mg. per cent, and those for mannitol between 100 and 200 mg. per cent. All solutions were prepared in 0.45 per cent saline solution. A Bowman constant injection pump delivered the solutions at an average rate of 1.90 ml. per minute, with a mean variation of 3.5 ± 2.5 per cent in the volume flow per minute.

Ascitic fluid was obtained in cirrhotic patients by means of an indwelling nylon catheter introduced into the peritoneal cavity through a 17-gauge needle.[20] Blood and ascitic fluid samples were obtained simultaneously at two, four, and eight hours during the mannitol infusion, and at eight and twenty-four hours during the inulin infusion. In patients with congestive heart failure, peripheral edema fluid was obtained by the subcutaneous insertion of 16 gauge needles specially prepared with numerous small holes bored along the barrel. Serial interstitial fluid and blood samples were simultaneously obtained as described above for ascitic fluid. The mannitol infusion was stopped after eight hours, while the inulin infusion was maintained for twenty-four hours. Blood samples were drawn at hourly intervals for three hours following the cessation of the mannitol infusion. Mannitol was infused for some six hours beyond the time required for equilibration in normal subjects,[13] whereas inulin was infused for eighteen hours after the six hours required for equilibration in normal subjects.[17] Inulin was determined by Harrison's[21] modification of the method of Alving, Rubin, and Miller.[22] Mannitol was determined by the method of Corcoran and Page.[23] In recovery experiments from plasma, inulin recovery averaged 98.7 ± 2.5 per cent, whereas the mannitol yield was 100.1 ± 6.5 per cent. The plasma concentrations of inulin and mannitol were corrected for water content on the basis of plasma protein determinations.

RESULTS

Ascitic fluid concentrations of inulin were significantly lower than those of plasma after twenty-four hours of continuous infusion (Table I). Likewise, inulin failed to equilibrate between plasma and interstitial edema fluid within a twenty-four hour period of infusion (Table II). The mean rate of equilibration in interstitial edema fluid appeared to be slower than in ascitic fluid. It was of interest to note that although inulin did not equilibrate after twenty-four hours, in four out of seven of the cardiac patients edema fluid was being mobilized, as was evidenced by obvious decrease in the amount of edema during the inulin infusion. Thus it would appear that the larger inulin molecule experienced difficulty in diffusing across the capillary endothelium while the smaller

TABLE I. RATES OF EQUILIBRATION OF INULIN BETWEEN PLASMA AND ASCITIC FLUID IN PATIENTS WITH CIRRHOSIS OF THE LIVER

	CONCENTRATIONS OF INULIN (MG. PER CENT) IN PLASMA WATER AND ASCITIC FLUID DURING A CONTINUOUS INTRAVENOUS INFUSION								
	8 HOURS				24 HOURS				TIME TO EQUILIBRATION (HOURS)
PATIENT	P*	A†	DIFF.‡	%§	P*	A†	DIFF.‡	%§	
1. V. G.	18.6	12.8	5.8	31.2	16.6	14.2	2.4	14.5	>24
2. N. N.‖	20.1	9.3	10.8	53.7	24.7	18.7	6.0	24.3	>24
3. J. K.	21.3	10.1	11.2	52.6	19.8	15.9	3.9	19.7	>24
4. C. R.	21.9	9.8	12.1	55.3	22.2	17.3	4.9	22.1	>24
Mean	20.5	10.5	10.0	48.2	20.8	16.5	4.3	20.1	>24
Standard deviation	—	—	2.8	11.4	—	—	1.5	4.2	—

*Plasma inulin concentrations.
†Ascitic fluid inulin concentrations.
‡Difference = P–A
§Per cent = per cent Difference = $\frac{P-A}{P} \times 100$
‖Patient markedly distended, jaundiced; very ill.

TABLE II. RATES OF EQUILIBRATION OF INULIN BETWEEN PLASMA AND PERIPHERAL EDEMA FLUID IN PATIENTS WITH CONGESTIVE HEART FAILURE

	CONCENTRATIONS OF INULIN (MG. PER CENT) IN PLASMA WATER AND EDEMA FLUID DURING A CONTINUOUS INTRAVENOUS INFUSION								
	8 HOURS				24 HOURS				TIME TO EQUILIBRATION (HOURS)
PATIENT	P*	E†	DIFF.‡	%§	P*	E†	DIFF.‡	%§	
1. M. F.‖	20.5	3.1	17.4	84.9	—	—	—	—	> 8
2. G. F.	21.1	4.4	16.7	79.1	23.8	16.3	7.5	31.5	>24
3. A. L.‖	14.1	3.3	10.8	76.6	13.9	9.2	4.7	33.8	>24
4. R. O.¶	22.9	5.2	17.7	77.3	—	—	—	—	> 8
5. P. G.¶	15.6	4.9	10.7	68.6	16.2	7.2	9.0	55.6	>24
6. A. A.‖	10.3	7.0	3.3	32.0	10.4	7.7	2.7	26.0	>24
Mean	17.4	4.6	12.8	69.7	16.1	10.1	6.0	36.7	—
Standard deviation	—	—	5.6	19.2	—	—	2.8	13.0	—

*Plasma inulin concentrations.
†Edema fluid inulin concentrations.
‡Difference = P–E
§Per cent = per cent Difference = $\frac{P-E}{P} \times 100$
‖Edema fluid rapidly subsided during infusion.
¶Patient went into acute failure during the infusion.

water molecules were diffusing out of the interstitial space. In contrast to inulin, mannitol diffused more rapidly into the ascitic fluid (Table III). Equilibration occurred between plasma and ascitic fluid water within eight hours. Mannitol failed to equilibrate with peripheral edema fluid within eight hours in a series of six patients with congestive heart failure (Table IV). In two patients, in which the mannitol infusion was continued beyond eight hours, one equilibrated within 9.3 hours, whereas the other required more than sixteen hours.

TABLE III. RATES OF EQUILIBRATION OF MANNITOL BETWEEN PLASMA AND ASCTIC FLUID IN PATIENTS WITH CIRRHOSIS OF THE LIVER

PATIENT	CONCENTRATION OF MANNITOL (MG. PER CENT) IN PLASMA WATER AND ASCITIC FLUID DURING A CONTINUOUS INTRAVENOUS INFUSION												TIME TO EQUILIBRATION (HOURS)
	2 HOURS				4 HOURS				8 HOURS				
	P*	A†	DIFF.‡	%§	P*	A†	DIFF.‡	%§	P*	A†	DIFF.‡	%§	
1. V. G.	93.7	74.7	19.0	20.3	90.4	88.7	1.7	1.9	89.9	98.2	−8.3	−9.2	5.1
2. N. N.	93.9	69.8	24.1	25.6	97.1	90.8	6.3	6.5	98.2	98.8	−0.6	−0.6	7.9
3. J. K.	114.5	63.8	50.7	44.3	111.3	97.8	13.5	12.1	109.1	115.8	−6.7	−6.1	7.4
4. C. R.	130.9	66.0	64.9	49.6	141.6	120.5	21.1	14.9	145.9	138.5	7.4	5.1	8.0+
Mean	108.2	68.6	39.7	34.9	110.1	99.4	10.7	8.8	110.8	112.8	−2.0	−2.7	7.1
Standard deviation	—	—	21.8	14.2	—	—	8.5	5.8	—	—	7.1	6.3	—

*Plasma manritol concentrations.

†Ascitic fluid mannitol concentrations.

‡Difference = P−A.

§Per cent = per cent difference = $\frac{P-A}{P} \times 100$.

TABLE IV. RATE OF EQUILIBRATION OF MANNITOL BETWEEN PLASMA AND PERIPHERAL EDEMA FLUID IN PATIENTS WITH CONGESTIVE HEART FAILURE

PATIENT	CONCENTRATION OF MANNITOL (MG. PER CENT) IN PLASMA WATER AND EDEMA FLUID DURING A CONTINUOUS INTRAVENOUS INFUSION																TIME TO EQUILIBRATION (HOURS)
	2 HOURS				4 HOURS				8 HOURS				16 HOURS				
	P*	E†	DIFF.‡	%§	P*	E†	DIFF.‡	%§	P*	E†	DIFF.‡	%§	P*	E†	DIFF.‡	%§	
1. M. F.‖	153.4	21.8	131.6	85.7	179.8	48.3	131.5	73.1	195.7	147.3	48.4	24.7	—	—	—	—	> 8
2. H. H.‖	124.3	37.8	86.5	69.6	145.9	48.0	97.9	67.1	—	—	—	—	—	—	—	—	> 4
3. G. F.	110.1	46.3	63.8	57.9	115.9	80.8	35.1	30.3	131.9	117.8	14.1	10.7	—	—	—	—	> 8
4. A. L.‖	57.4	10.3	47.1	82.1	62.2	18.8	43.4	69.8	68.2	44.3	23.9	35.0	—	—	—	—	> 8
5. R. O.¶	120.3	32.3	88.0	73.2	130.0	73.3	56.7	43.6	150.5	108.8	41.7	27.7	—	—	—	—	> 8
6. P. G.¶	—	—	—	—	62.1	44.9	17.2	27.7	78.2	47.9	30.3	38.7	97.6	88.9	8.7	8.9	>16
7. A. A.‖	—	—	—	—	71.0	62.4	8.6	12.1	69.9	63.4	6.5	9.3	62.4	76.4	−14.0	−22.4	9.3
Mean	113.1	29.7	83.4	73.7	109.5	53.8	55.8	46.2	115.7	88.2	27.5	24.3	80.0	82.7	− 2.7	− 6.7	—
Standard deviation	—	—	31.8	11.0	—	—	44.7	24.1	—	—	16.0	12.2	—	—	—	—	—

*Plasma mannitol concentrations.
†Edema fluid mannitol concentrations.
‡Difference = P–E.
§Per cent = per cent difference = $\frac{P-E}{P} \times 100$.
‖Edema fluid rapidly subsided during infusion.
¶Patient went into acute failure during the infusion.

DISCUSSION

In the determination of the inulin space by the infusion-recovery method and the mannitol space by the infusion-decrement method, it is necessary that equilibration has occurred at the time that the infusion is stopped. Since mannitol and inulin are of grossly different molecular sizes, one might expect these solutes to diffuse into the interstitial phase at different rates. One might also expect that these solutes would diffuse into various types of edema fluid at different rates. The data reported in this paper support both of these assumptions. As was to be expected, mannitol* diffused more rapidly than inulin† into both ascitic fluid and peripheral edema fluid. After twenty-four hours of continuous infusion, inulin failed to equilibrate with either ascitic fluid or peripheral edema fluid, whereas mannitol equilibrated between plasma and ascitic fluid in less than eight hours. In striking contrast, mannitol failed to equilibrate within eight hours or for that matter even within sixteen hours between plasma and peripheral edema fluid.

In patients with ascites of long duration, one might assume that such ascitic fluid would be relatively adynamic in contrast to peripheral edema fluid which can be mobilized in patients with congestive failure. The data indicate that both inulin and mannitol experienced greater difficulty in diffusing through peripheral capillary beds into the interstitial phase of the lower limbs than across the peritoneal capillaries into ascitic fluid.

SUMMARY

Inulin and mannitol equilibrated at a faster rate across the peritoneal capillaries in cases of cirrhosis with ascites than across the peripheral limb capillaries in cases of congestive heart failure with dependent edema.

The continuous intravenous infusion of mannitol in patients with ascites resulted in equilibation in approximately eight hours. Mannitol failed to equilibrate between plasma and peripheral edema fluid following eight hours of continuous infusion in a series of patients with congestive heart failure.

Inulin failed to equilibrate between plasma and interstitial water even after twenty-four hours of continuous infusion in patients with cirrhosis with ascites and congestive heart failure with peripheral edema. The determination of the inulin space in edematous patients by the infusion-recovery method would appear to be impractical in view of the failure to equilibrate within a reasonable period of time.

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*Mannitol-molecular weight, 182; diffusion coefficient, 0.50 × 10^{-5} $cm.^2$/sec. at 20° C.[24]
†Inulin-molecular weight, 5,100; diffusion coefficient, 0.16 × 10^{-5} $cm.^2$/sec. at 20° C.[24]

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PHYSIOLOGICAL CHANGES DURING PERFUSION OF THE ISOLATED INTESTINAL LOOP IN CHRONIC UREMIA

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THOUGH many procedures have been devised in attempts to tide patients over periods of acute renal failure, relatively few reports have dealt with attempts to design methods for treatment over extended periods of chronic renal insufficiency. Only within the past few years has perfusion of isolated segments of the bowel[1-3] or of the intact gastrointestinal tract[4-9] been used to try to alleviate the clinical effects of chronic renal failure. All methods have succeeded in lowering blood urea levels, but difficulties have been encountered with blood level changes of various electrolytes; particularly the sodium, potassium, and calcium ions. There is also evidence[10, 11] that ion movements across the intestinal membrane vary at different levels of the gut. At the ileal level there have been few studies on the electrolyte composition of the succus entericus,[12, 13] and there are no reports of urea extraction, fluid balance, and ion exchange in the ileum of patients with chronic renal disease.

In this paper observations of electrolyte, urea, and fluid exchange during attempts to treat two patients terminally ill from chronic uremia by perfusion of an isolated ileal loop are presented. The physiologic implications of the results, and the possible theoretical advantage of the choice of a segment of the intestinal tract other than the terminal ileum for perfusion are discussed in the light of the complications and obstacles to therapeutic success that were encountered.

The two patients studied had chronic renal insufficiency of long standing. In one (J. J.) uremia was secondary to renal amyloidosis, in the other (M. H.) it was caused by polycystic kidney disease. In each patient approximately six feet of the terminal ileum was isolated, with its blood supply intact, and perfused after the ends of the loop were brought out through incisions in the lower abdominal wall. Both patients died. It cannot be stated unequivocally that the procedure prolonged life.

PROCEDURE

Operation.—Both patients† were operated under spinal anesthesia, the ileum being visualized through a midline abdominal incision. The ileum was transected at two points, 8-2 inches and 10 inches, proximal to the ileocecal valve; the mesentery was left intact. The

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†The surgery was performed by Dr. Dwight E. Clark.

proximal and distal bowel were reanastomosed. The ends of the isolated ileal loop were brought out through incisions made in the upper portion of each lower abdominal quadrant. The abdominal incision was closed. In each case the operative procedure was uneventful.

Perfusion in patient J. J. was started thirty hours postoperatively and continued for ninety-six hours until death. In patient M. H., the perfusion was started thirty hours postoperatively and carried out intermittently during the thirty-one days following operation for periods varying from eight to forty-eight hours.

Apparatus and Perfusion Technique.—The apparatus used in perfusing the isolated ileal loop is presented in Fig. 1. The perfusing solution was introduced into the proximal end of the isolated loop via a soft rubber catheter inserted approximately 15 cm. to prevent excessive back flow from rapid rates of flow. Leaking at the ileostomies was minimized by attaching condoms over the ends of the protruding bowel. Fluid was collected from the distal end of the loop by a wide (1.0 cm. I.D.) semi-rigid rubber tube inserted into the tip of the condom. Flow through the loop had an intermittent constancy as a result of the peristaltic activity of the bowel.

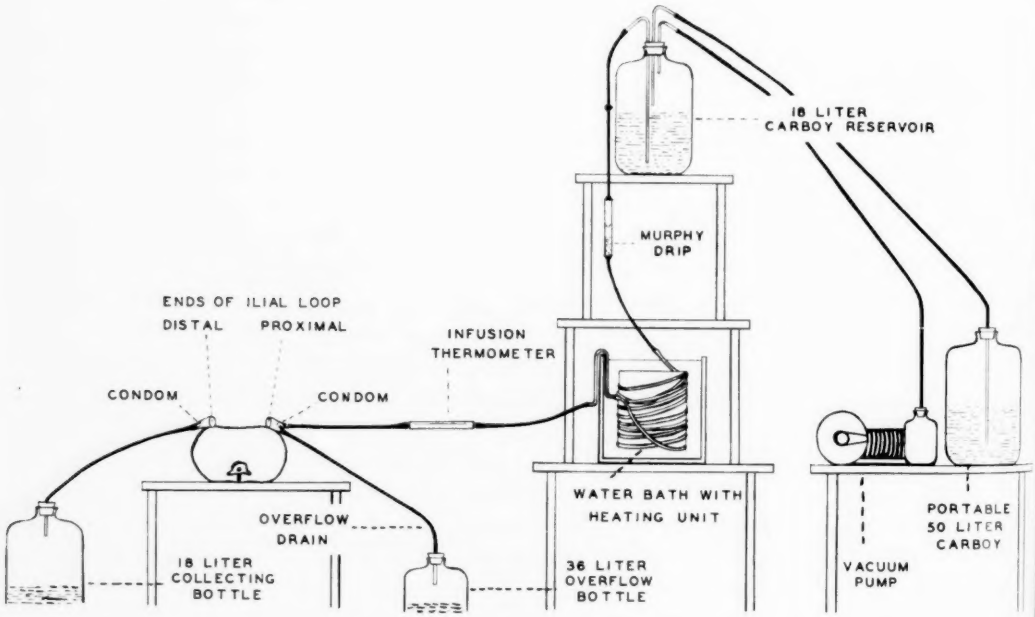

Fig. 1.—Diagram of apparatus for perfusion of an isolated intestinal loop.

When the perfusing fluid was altered as to be free of a particular substance, the reservoir was rinsed with distilled water before starting the fluid. A minimum of 5 liters of each special solution was allowed to pass through the loop before a sample was taken from the collecting bottle.

Composition of Perfusing Fluids.—The perfusion fluids used are shown in Table I. Solution I was essentially the same structure as the "P" solution of Odel and Ferris[14] and the peritoneal lavage solution of Kolff.[1] Calcium gluconate was used in Solution II to raise the calcium concentration without adding further chloride. During several phases of the study, Solution II was so altered that it was free of potassium or calcium and phosphate. Distilled water was used throughout.

TABLE I

SOLUTE	SOLUTION I GM./L.	SOLUTION II GM./L.
A. Structure of Perfusing Solution I (Patient J. J.) and Solution II (Patient M. H.)		
Sodium Chloride	6.0	6.0
Potassium Chloride	0.2	0.4
Calcium Chloride	0.28	-
Calcium Gluconate	-	1-5
Magnesium Chloride	-	0.1
Sodium Acid Phosphate	0.05	0.05
Sodium Bicarbonate	3.0	3.0
Dextrose	20-15	30-15
B. Ionic Composition of Perfusing Solutions		
Sodium (meq/L.)	139.5	139.5
Potassium (meq/L.)	2.69	5.38
Calcium (mg. %)	10	10-50
Phosphate (mg. %)	4	4
Magnesium (mg. %)	-	0.25
Chloride (meq/L.)	110.3	109
Bicarbonate (meq/L.)	35.8	35.8

METHODS

Analyses of blood, perfusing fluid, and perfusate were performed as follows: urea nitrogen by the Van Slyke gasometric method;[15] calcium, magnesium, and phosphorus by the modified methods of Eichelberger and Bibler;[16] sodium and potassium by the Beckman flame photometer;[17] glucose by the Shaeffer-Hartman-Somogyi procedure;[18] serum carbon dioxide content by the method described by Van Slyke and Neill;[19] serum pH by Cullen's method;[20] and chloride by the method of Schales and Schales.[21] Blood samples were drawn each morning at approximately 8:00 A.M., and in the case of J. J. at other times as indicated. Aliquot samples of perfusing fluid were taken from the collecting bottle after thorough mixing.

CASE HISTORIES OF THE PATIENTS REPORTED

CASE 1.—J. J. (416193). This 29-year-old white man was admitted for the second and last time to Billings Hospital in October, 1948. His history revealed that he had a draining sinus due to osteomyelitis of the left hip from 1939 to 1945. He developed a sore throat late in 1946 followed shortly by severe occipital headaches, vomiting, elevated blood pressure readings, and albuminuria. In 1947 his blood pressure was 180/120. Urinalysis revealed fatty casts, white blood cells, and double refractile fat bodies. A diagnosis of chronic glomerulonephritis with secondary hypertension was made; renal amyloidosis secondary to the osteomyelitis was considered an improbable second diagnosis.

This patient was admitted to the hospital for the first time in September, 1948, because of exertional dyspnea, easy fatigability, and a sore throat. Blood pressure was 200/130; optic fundi were not remarkable; heart and lungs were clear; the liver was not palpable; and there was pitting edema. Urinalysis showed a 3 plus albuminuria and white blood cells. Hemoglobin was 6 grams. A throat culture grew out beta-hemolytic streptococcus. He received transfusions and antibiotics and, feeling better, signed himself out of the hospital.

At home he developed severe nausea, vomiting, paroxysmal nocturnal dyspnea, and nose bleeds. In October, 1948, at the patient's request, he was readmitted to the hospital. Blood pressure was 185/142; the lungs were clear; the left border of cardiac dullness was 12 cm. in the fifth interspace; a firm, sharp, and nontender liver edge was palpable 2 cm. below the right costal margin. There was 2 plus pitting edema of the lower extremities. Urinalysis revealed specific gravity 1.005, a 3 plus albuminuria, red cells, and numerous hyaline and granular casts. Hemoglobin was 12.5 Gm., and red blood cell count 3.8 million. The blood urea nitrogen was 114.7 mg. per cent; serum CO_2 was 19.2 mM per liter; serum chloride 96.8 mM per liter; plasma protein 6.94 Gm. per cent (albumin 3.3 Gm. per cent and

globulin 3.63 Gm. per cent); serum calcium 8.3 mg. per cent; and serum phosphorus 11 mg. per cent. Phenolsulfonphthalein excretion in one hundred twenty minutes was 1.75 per cent. Nose and throat cultures were negative for beta-hemolytic streptococcus. The electrocardiogram showed left ventricular strain.

Following admission, his blood urea nitrogen steadily rose, and he continued nauseated and vomiting until November 11. On November 19, the patient's sensorium was clouded. He was placed on exsanguinating transfusions which reduced his blood urea nitrogen from 131.3 mg. per cent to 119.3 mg. per cent. On November 28, he developed a friction rub. The patient rapidly began to deteriorate, being unable to recognize people or objects around him. On December 13, he was taken to surgery and a loop of ileum was isolated. The next day perfusions were started through this loop.

On December 16, the patient was responsive and able to read a newspaper, but on December 18, he responded poorly. His ankles and elbows were edematous, the heart tones were distant, and there was a pericardial effusion. By 10 P.M. he was in frank tetany. Intravenous calcium gluconate gave little relief, even in huge doses. Magnesium sulfate did not stop the tetanic convulsions. He became apneic and died several minutes later at 1:20 A.M. Autopsy revealed generalized amyloidosis.

CASE 2.—M. H. (431325). This 45-year-old white man entered Billings Hospital for the fifth and last time on March 4, 1949. His previous hospitalizations, dating from April, 1948, had revealed bilateral polycystic kidney disease with hypertension, anemia, cardiomegaly, hepatomegaly, renomegaly, cardiac failure, acidosis, hypocalcemia, hypertensive retinopathy, and uremia.

His previous therapy included numerous blood transfusions, a low protein diet, antibiotics when needed, calcium gluconate, aluminum hydroxide, and digitalis.

The interval between hospitalizations decreased, and it was only one month after his previous hospital discharge that he was admitted for the last time.

Physical examination revealed a thin, stuporous white man. Blood pressure was 210/110; in the fundi the discs were well outlined; the A-V ratio was 1:2 peripherally and 2:3 centrally; there was a very slight A-V nicking with a moderate widening of the light reflex; there were two small hemorrhages in the left nasal field, but no exudates. The breath was uremic. There was moderate costal vertebral angle tenderness. There were bilateral basilar moist râles in the lungs. Left border of cardiac dullness was 10.5 cm. in the fifth interspace. A-2 was greater than P-2. There was a short, harsh, decrescendo, low-pitch basal murmur heard best over the aortic area and transmitted to both shoulder regions. The abdomen was distended. There was a regular, firm, tender liver edge four fingerbreadths below the costal margin. The kidneys were markedly enlarged, firm, irregular, and exquisitely tender.

Urinalysis showed a specific gravity of 1.007, 2 plus albumin; 4 celiular casts, 1 to 2 red blood cells, and 6 to 10 white blood cells per high-power field. The red blood count was 2.5 million; the hemoglobin was 7.25 Gm.; white blood count was 10,500. Stools were negative for blood; the blood urea nitrogen was 194 mg. per cent; the blood nonprotein nitrogen was 215 mg. per cent; the serum CO_2 10.5 mM per liter; the serum chloride was 107.5 mM per liter; pH 7.13; serum calcium 5.8 mg. per cent; serum phosphorus 10.5 mg. per cent; total plasma protein 6.76 Gm. per cent (albumin 1.56 Gm. per cent and globulin 5.2 Gm. per cent). Urea clearance by standard formula was 1 ml. per minute.

He was treated intensively, and on March 15 a loop ileostomy was constructed. On March 16, perfusion through the loop was started. By March 23, he was sitting up in a chair, and walking. He was kept on intravenous calcium gluconate as muscular twitchings and complaints of numbness appeared to be the most pressing problem. He began complaining bitterly of epigastric burning, and had continuous green-black involuntary bowel movements, all benzidine positive. He became less alert; his temperature began to rise, and twitchings of the extremities became more marked. Though grams of urea were being removed by way of the intestinal loop, his blood urea nitrogen began to rise. By April 13 he had been unresponsive for thirty-six hours. His respirations became more labored, and he expired at 12:45 A.M. on April 14.

Autopsy revealed the heart to be only slightly enlarged; there was a very sparse pneumonia. Many of the renal cysts bilaterally were infected and there was little recognizable kidney tissue to be seen. There was an ulcerative cystitis. Numerous small bleeding points were present in the intact gastrointestinal tract, but in the ileal loop no bleeding points were seen. The mucosa of the loop was microscopically normal.

RESULTS

In patient M. H. only two perfusion periods were observed in which vomiting and intestinal bleeding did not complicate the procedure. Data obtained during Period I (the day preceding operation to the third postoperative day)

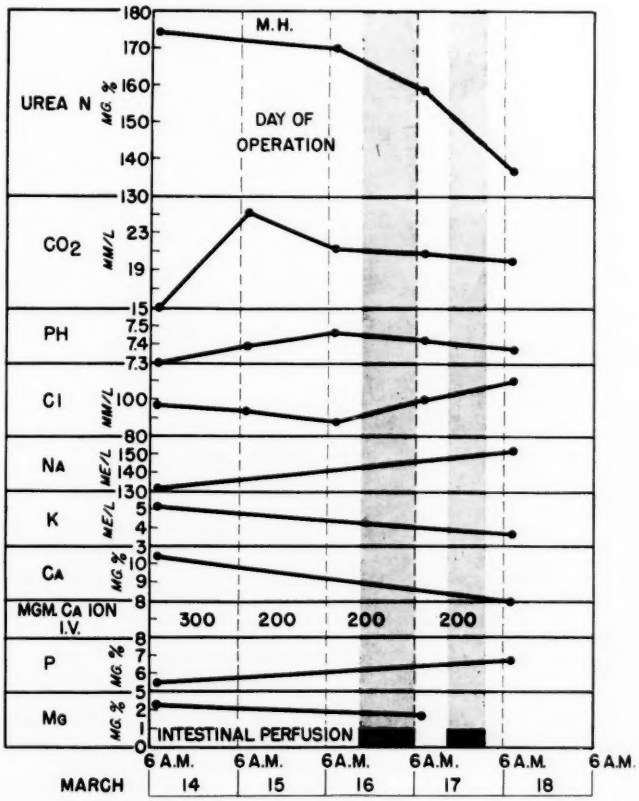

Fig. 2.—Changes in chemical constitution of blood serum before and after perfusion of an intestinal loop and administration of intravenous calcium during Period I of patient M. H. The gray vertical bars with black bases represent duration of perfusion.

and during Period II (nineteenth to the twenty-third postoperative days) are presented in Figs. 2 and 3, respectively. Data on fluid balance, urea excretion, and electrolyte exchange within the intestinal loop are taken from these and from other perfusion periods.

The data obtained in patient J. J. are presented in Fig. 4.

Fluid Balance in the Intestinal Loop.—The volumes of fluid introduced into and recovered from the loop in patient M. H. with the variables of glucose

concentration and rate of flow are presented in Table II. The volumes recovered were essentially equal (± 3 per cent) to the volume introduced into the loop.

In patient J. J., 132 liters of fluid were introduced into the loop during ninety-six hours of perfusion, and 125 liters were recovered. Leakage decreased the accuracy of this latter value.

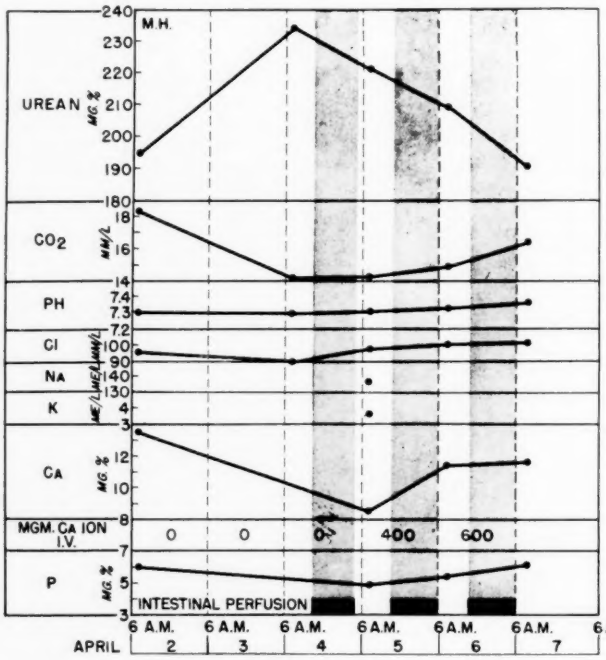

Fig. 3.—Changes in chemical constitution of blood serum before and after perfusion of an intestinal loop and administration of intravenous calcium during Period II of patient M. H. The gray vertical bars with black bases represent duration of perfusion.

TABLE II. FLUID BALANCE IN THE ISOLATED ILEAL LOOP. PERFUSION SOLUTIONS CONTAIN VARYING CONCENTRATIONS OF GLUCOSE AT VARYING RATES OF FLOW*

DATE	HOURS OF PERFUSION	RATE OF FLOW (C.C./MIN.)	GLUCOSE (GM./L.)	PERFUSING SOLUTION: LITERS INTRODUCED	PERFUSING SOLUTION: LITERS RECOVERED	PERFUSING SOLUTION: LITERS/HR. INTRODUCED	PERFUSING SOLUTION: LITERS/HR. RECOVERED	NET/HR.
3/16	14.5	24	25	20.5	21.2	1.41	1.46	−0.05
3/17	10	33	25	19.5	20.2	1.95	2.02	−0.07
3/23	8.5	20	20	10.4	10.37	1.22	1.22	±0.00
			15					
3/29	7.5	33	15	22.9	22.7	3.05	3.03	+0.02
4/ 1	2.5	71	30	10.6	10.3	4.24	4.12	+0.12
4/ 5	5	111	25	33.4	34.1	6.68	6.82	−0.14
4/ 7	3.5	81	25	17	17	4.86	4.86	±0.00
4/ 9	10	60	25	35.7	34.3	3.57	3.43	+0.14
4/10	21	61	20	77.3	77.6	3.68	3.70	−0.02

*All data presented are from patient M. H.

The following data, except when specifically indicated, were obtained from patient M. H.

NONELECTROLYTES

Urea.—Blood urea nitrogen was consistently lowered by perfusion. The average time of the five perfusions during Periods I and II was fourteen hours (ten to sixteen hours). The average blood urea nitrogen concentration decrease following each perfusion was 15.3 mg. per cent (11.5 to 21.1 mg. per cent).

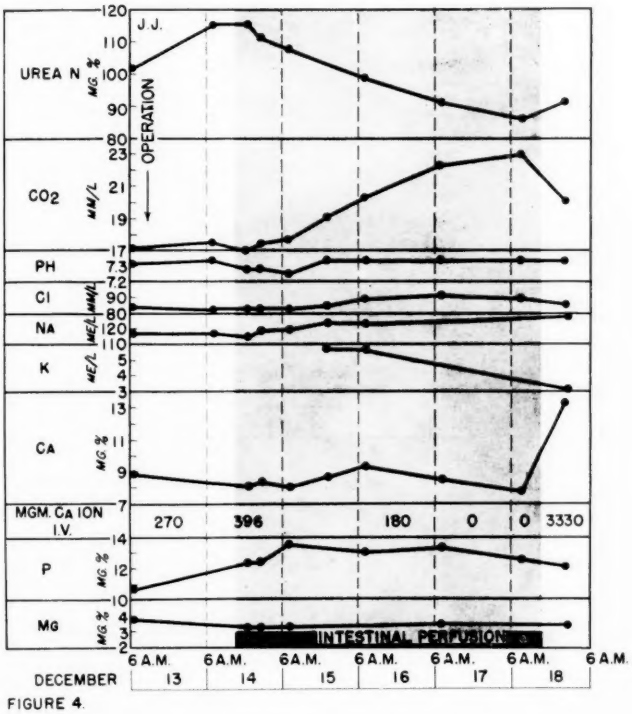

FIGURE 4.

Fig. 4.—Changes in chemical constitution of blood serum before, during, and after perfusion of an intestinal loop and administration of intravenous calcium in patient J. J. The gray vertical bar with black base represents duration of perfusion.

As shown in Fig. 5*A*, the concentration of urea nitrogen in the perfusion fluid recovered from the loop varied inversely with the rate of flow of the perfusing solution. The maximum concentration obtained was 82 mg. per cent from a flow of 29 ml. per minute; the minimum was 31.1 mg. per cent from a flow of 123 ml. per minute. Total urea nitrogen extracted per hour ranged from 1.15 to 3.08 Gm. This value varied directly with the flow as indicated in Fig. 5*B*. Blood urea clearance $\left(\frac{UV}{B}\right)$ through the loop is presented in Fig. 5*C* and varied from 8 to 35 ml. per minute, with an average of 20.6 ml. per minute. In patient J. J., the blood urea nitrogen level decreased from 115 mg. per cent

to 86 mg. per cent during ninety-six hours of perfusion (Fig. 4). Blood urea clearance through the loop varied from 5 to 10 c.c. per minute, with an average of 7.5 c.c. per minute.

Glucose.—The blood glucose concentration was 189 mg. per cent after six hours of perfusion with a fluid containing 25 Gm. of glucose per liter. On another day, the blood glucose level was 181 mg. per cent nine hours after the completion of an intravenous infusion of 1,500 ml. of 5 per cent glucose in distilled water when there was no perfusion. Therefore, glucose was apparently absorbed from the loop during perfusion.

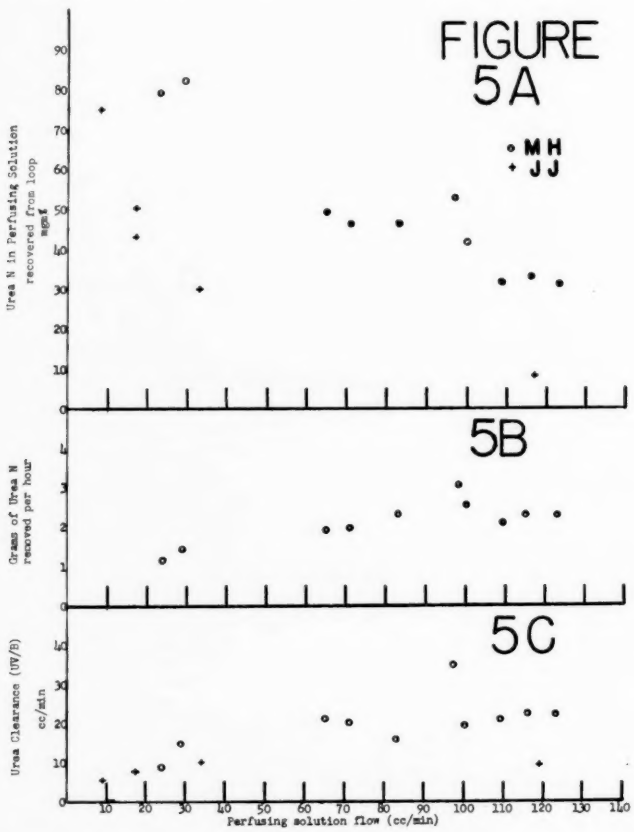

Fig. 5.—The relationship between removal of urea nitrogen by the intestinal loop and the rate of perfusion of fluid through the loop (+, patient J. J.; o, patient M. H.).

ELECTROLYTES

Carbon Dioxide and Hydrogen Ion Concentration.—In Period I the serum carbon dioxide content rose from 15.2 to 25.2 mM per liter, and the serum pH from 7.30 to 7.46 as a result of intensive preoperative alkali therapy. During perfusion, the carbon dioxide content remained essentially unchanged (decreased from 21.3 to 20.0 mM per liter) and the pH fell to 7.37. During Period II the

TABLE III. THE EFFECT OF PASSAGE THROUGH THE ISOLATED ILEAL LOOP ON THE ELECTROLYTE CONCENTRATIONS OF THE PERFUSION SOLUTION

	BEFORE PERFUSION						AFTER PERFUSION					
FLUID	NA MEQ./L.	K MEQ./L.	CL MEQ./L.	CA MG. PER CENT	P MG. PER CENT	MG MG. PER CENT	NA MEQ./L.	K MEQ./L.	CL MEQ./L.	CA MG. PER CENT	P MG. PER CENT	MG MG. PER CENT
1				9.86	0.84	0.99				10.6	0.79	0.88
1				9.86						11.05		
2A				0						1.94		
2				17.5						16.35		
2				17.5						17.8		
3				16.5						17.95		
3				16.9	1.07					17.75	1.06	
4	138.2	5.65					135.5	5.88				
6	140.8	5.95					128.8	6.45				
7	140	6.3	107.0	0.0			132.0	6.45	107.1	0.85		
7	140	6.3		0.0			134.6	6.8		0.3		
8	140	0.0					133.2	Trace				
9			107.9	50.2	1.05				107.1	50.6	1.0	
10			0.04	0.0	0.0				4.16	1.4	0.1	
11			109.5	34.5	0.99				109.1	33.8	1.09	
11				34.5	0.99					35.4	1.32	

*All data presented are from patient M. H.

carbon dioxide content increased from 14.1 to 16.4 mM per liter and was accompanied by a rise in pH from 7.29 to 7.36. Patient J. J. demonstrated an increase in carbon dixoide content from 16.9 to 23.0 mM per liter and a pH increase from 7.28 to 7.34.

Chlorides.—In all cases (Figs. 2, 3, and 4), there was an increase of serum chloride level during perfusion. The rise from 88 to 110 mM per liter following 24.5 hours of perfusion in Period I (Fig. 2) was the greatest observed. The increase in Period II was from 89 to 101 mM per liter, and in patient J. J. from 84.4 to 91.2 mM per liter.

Chloride concentration in the perfusing fluid was not significantly affected by passage through the loop (Table III). An essentially chloride-free perfusing fluid (0.04 mM per liter) had a chloride concentration of 4.16 mM per liter after perfusion.

Sodium.—Serum sodium levels increased during perfusion. In Period I (Fig. 2), it rose from 131.8 to 152.8 meq. per liter. About one-half of this rise is estimated to be due to the preoperative alkali therapy. In patient J. J., there was an increase in serum sodium from 117 to 129.4 meq. per liter.

The sodium concentration of the perfusing fluid decreased during passage through the loop. As shown in Table III, this decrease ranged from 2.5 to 12.0 meq. per liter.

Potassium.—Serum potassium concentration decreased during perfusion. In Period I, the level fell from 5.1 to 3.7 meq. per liter. In patient J. J., the decrease was from 5.7 to 3.1 meq. per liter. The potassium concentration of the perfusing fluid increased during perfusion. As shown in Table III, this increase ranged from 0.15 to 0.5 meq. per liter.

Calcium.—Serum calcium concentration decreased during perfusion in Periods I and II and in patient J. J. (Figs. 2, 3, and 4). This progressed to levels producing tetany unless replaced by intravenous calcium gluconate. Approximately 500 mg. of calcium ion (as calcium gluconate) were necessary to maintain the serum calcium level while the patient was undergoing perfusion lasting twelve hours.

The calcium concentration in the perfusing fluid varied from 0.0 to 50.2 mg. per cent (Table III). In ten of twelve samples analyzed the concentration was increased following perfusion. The average increase for all twelve samples was 0.60 mg. per cent (range—1.15 to +1.94 mg. per cent). Addition of vitamin D_2 in propylene glycol* 1,000 units per liter and bile salts† 100 mg. per liter did not produce calcium absorption.

Phosphorus.—Serum inorganic phosphate levels followed no consistent trend attributable to perfusion (Figs. 2, 3, and 4). Phosphorus concentrations in the fluid revealed small inconstant changes following perfusion (Table III). Excretion of phosphorus through the wall of the loop was demonstrated. 0.5

*Drisdol, Winthrop-Stearns, Inc.
†Bilein, Abbott Laboratories.

mc.c* of radioactive phosphorus (as Na_2HPO_4) was injected intravenously.† Ten liters of fluid were passed through the loop during the four hours following injection, and were found to contain 0.023 per cent of the P_{32} administered.

Magnesium.—In patient J. J., there was no significant change in the serum magnesium level after perfusion with magnesium free fluid lasting ninety-six hours.

Bleeding.—The hemorrhagic tendencies of uremic patients are well known. In patient M. H. there was a continuous oozing of blood into the reanastomosed gastrointestinal tract the last few days of life. During this same period of time, there was not evidence, either microscopic or chemical, that bleeding was taking place in the ileal loop. At autopsy no bleeding points or areas were seen in the mucosa of the perfused ileum.

DISCUSSION

Though several investigators[1, 2, 4, 6, 8] within recent years have perfused the small bowel in an attempt to treat chronic uremia, there has been little or no data available as to fluid balance and ion exchange between the blood and perfusing solutions. Particularly is this true of the calcium and potassium ions. The normal value quoted[13, 22, 23] for calcium in the ileum succus entericus is derived from one determination on one dog.[12]

The data obtained in this study give some indication of what can be anticipated from intermittent perfusion of the isolated ileal loop in chronic uremia with the dynamic changes in fluid balance, urea clearance, and ion exchange.

Fluid Balance.—It has been the experience of others that fluid balance could be controlled by adjusting the tonicity of the perfusing fluid with varying concentrations of glucose. Such an effect was not consistently demonstrated in the ileal loop in patient M. H. The perfusing fluid appeared to behave as an isotonic solution regardless of the glucose concentration, except for Period I when the fluid containing 25 Gm. of glucose per liter acted in a hypertonic manner and water was withdrawn from the patient. Subsequently, although the glucose concentration of the fluids was varied from 15 to 30 Gm. per liter, the volumes of solution introduced into and recovered from the loop were nearly identical.

On the other hand, an effect apparently related to altered glucose concentration was noted in patient J. J. With a glucose concentration of 20 Gm. per liter in the perfusing fluid, edema gradually developed. After reduction to 15 Gm. per liter, the edema accumulated more rapidly.

Urea.—Present concepts are that urea is relatively nontoxic, as the previously experimentally demonstrated toxicity has been attributed to secondary effects of the substance.[24] It has been shown by Pendleton and West[25] that in dogs an isotonic solution placed in an isolated loop of small bowel will obtain a urea nitrogen concentration identical with the blood level. These workers

*Curie—amount of radium emanation in a closed container in radioactive equilibrium with one gram of radium element.

†We are indebted to Dr. William F. Bethard for the radioactive phosphorus study.

postulate that the gut wall acts as a semipermeable membrane for this substance. It has repeatedly been demonstrated that urea can be removed through the intestinal wall of man.[6, 7] A normal man on a high protein diet excretes approximately 16.8 Gm. of urea nitrogen in the urine in twenty-four hours.[26] In patient M. H., if perfusion at a flow of 100 ml. per minute was maintained for eight hours, approximately 20 Gm. of urea nitrogen would have been extracted. While the efficiency of the loop in removing urea would be anticipated to be less with lower blood urea nitrogen levels than were present in this study, it appears that a negative urea balance can be maintained by even short intermittent perfusions. Data are insufficient to conclude whether the apparent increase in urea clearance with increase in rate of flow of perfusion fluid in patient M. H. is real.

Sodium.—Serum sodium concentrations increased during perfusion, and the perfusing fluid sodium concentration decreased after passage of the fluid through the loop of terminal ileum. In Period I in patient M. H. and in patient J. J., the serum sodium levels at the beginning of the perfusion periods were lower than the perfusing fluid sodium levels. This difference suggests that the increase in serum sodium concentration during perfusion could result from the simple diffusion of sodium through the bowel wall. However, during a perfusion of patient M. H., not included in Periods I or II, the serum sodium concentration at the beginning of the perfusion period was higher than the perfusing fluid sodium concentration. Nevertheless, the perfusing fluid sodium level still decreased after passage through the loop. On this occasion, the serum sodium was 146 meq. per liter. The solution used contained 140 meq. per liter before perfusion and 134 meq. per liter after perfusion. This observation suggests that sodium absorption is by a mechanism other than simple diffusion.[22] A similar situation has been found to exist in the colon by Daugherty and associates,[7] who suggest that the perfusing fluid be hypotonic in sodium or even sodium free. The studies of Visscher and associates[10, 11] on dogs using radioactive sodium indicates that there is a greater absorption of this ion the more distal the intestinal segment, while excretion of sodium is lessened. This suggests that the use of a proximal jejunal loop might result in better control of sodium exchange.

The acidosis present in both patients was improved during perfusion. This is attributed to the absorbed sodium producing an increase in available base.

Potassium.—In both patients serum potassium levels and electrocardiograms revealed no evidence of potassium abnormality. Following perfusion there was a reduction in serum potassium concentration with an accompanying increase in the concentration of potassium in the perfusing fluid. These changes occurred with the potassium concentration in the perfusing fluid higher than in the serum, and suggests that potassium excretion is by mechanisms other than simple diffusion. Similar findings were noted by Bernstein and associates,[3] who had perfused the stomach, and Hicks,[27] who has utilized gastric washing as a therapeutic procedure for reducing high blood potassium levels.

Because the potassium ion loss, unlike the calcium ion loss, did not have clinical manifestations, the perfusing solutions were not altered in composition to attempt to produce higher potassium ion concentration in the serum.

Calcium and Phosphorus.—Hypocalcemia and hyperphosphatemia are frequently found in chronic uremia, and were present in both patients studied. Intestinal perfusion further aggravated this calcium-phosphorus imbalance, as calcium was continually extracted in spite of concentrations of calcium in the perfusing fluid up to 50.2 mg. per cent. If the perfusion was maintained without adequate intravenous calcium replacement, tetany developed. The addition of vitamin D and bile salts to the perfusion fluid had no apparent effect upon calcium absorption.

Calcium is supposedly absorbed in the duodenum and excreted in the gastrointestinal tract by the mucosa of the large bowel.[28] The jejunum and ileum have not specifically been implicated in calcium metabolism. In both of these patients where only the terminal six feet of small bowel were used for perfusion, there is evidence that calcium is excreted by the mucosa of the ileum. If a loop of duodenum and jejunum had been utilized instead of terminal ileum, hypocalcemia might not have developed. Calcium balance might also have been achieved if the food intake had been adequate.

Perfusion had no apparent direct effect on the serum inorganic phosphate level. The increase observed during perfusion may be related to the decrease in the serum calcium concentration. Changes in the phosphate concentration of the perfusing fluid after passage through the loop were inconclusive. There was some phosphate excretion into the loop, but this occurred only in trace amounts as measured by P_{32}.

Bleeding.—Fishberg[29] postulates that the intestinal bleeding seen in uremics may well be due to the breakdown of urea into ammonia by bacteria, and that the ammonia acts on the mucosa causing ulcerations and bleeding. It may be that the solution running through the ileal loop washed away or lessened the concentration of some toxic product which is responsible for the hemorrhage. Another hypothesis is that the perfusing solution contains some element, perhaps calcium, which is needed in higher concentrations to keep the clotting mechanisms intact.

SUMMARY AND CONCLUSIONS

Two terminally ill chronic uremic patients with ileal loops 6 feet in length were studied during perfusion of these loops. The following biochemical changes occurred:

In the Loop.—The volumes of fluid introduced and recovered were essentially (±3 per cent) equal. The highest amount of urea nitrogen extracted was 3.08 Gm. per hour, and the loop urea clearance varied from 5 to 35 ml. per minute. Glucose was absorbed. The concentration of chloride was not significantly changed in passage of perfusate through the loop, sodium concentration decreased, and calcium and potassium concentrations increased. Phosphorus showed small inconstant changes, though an intravenous injection of radioactive phosphorus revealed excretion into the intestine.

Bleeding was present in the reanastomosed bowel; there was no evidence, microscopic or chemical, of hemorrhage in the perfused ileal loop.

In the Blood.—During perfusion the concentrations of carbon dioxide, chloride, and sodium increased; calcium and potassium decreased; and inorganic phosphorus was unchanged. The urea concentration in the blood was decreased.

It is doubtful if the ileum is the most suitable anatomic location for a loop to serve effectively in the relief of the biochemical manifestations of uremia. In the two patients studied, it was impossible to supply enough calcium and potassium to raise blood levels via the perfusing solution because excessive secretion of both potassium and calcium took place even against high concentration gradients. In contrast, sodium was readily absorbed, the serum sodium gain and calcium loss were of clinical significance in these patients, and were manifested by edema and hypocalcemic tetany requiring intensive therapy. The potassium loss was not severe enough to cause clinical symptoms.

Reduction of the blood urea concentration can be accomplished by perfusion of an ileal loop, but it is obvious that in order to have practical value of combatting chronic uremia, means must be found to correct the profound disturbances in electrolyte balance that may result from perfusion of the loop. From recent work of other investigators performed on dogs[2, 10, 11] the jejunum appears to be a more logical location for an intestinal loop because greater absorption and sodium excretion take place at this intestinal level.

ADDENDUM

Since the preparation of this paper, Twiss, and Kolff[30] have published a report on perfusion of an isolated intestinal loop of mid-gut 2.5 meters long in a terminal patient. Their findings imply that at the higher intestinal level sodium and potassium blood and loop changes were similar to those at the terminal ileum level, but that calcium blood levels could be controlled by changes in the dialyzing fluid.

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RIBOFLAVIN EXCRETION DURING PREGNANCY AND EARLY LACTATION

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THE requirement of various vitamins is greatly increased during pregnancy and lactation, and markedly exceeds that of nonpregnant individuals. Various manifestations of vitamin deficiency are therefore more likely to occur during these periods of human life[1, 2] than at other times.

In previous papers on riboflavin deficiency,[3-5] we were able to demonstrate that riboflavin deficiency manifestations frequently occur in pregnant women, and that their frequency increases with the progress of the pregnancy. During the periods of dietary restrictions in Israel, 21 per cent of pregnant women were found to be suffering from ariboflavinosis. Other healthy members of these patients' families living under identical dietary conditions generally exhibited no signs of deficiency. This fact strongly suggests that the riboflavin requirements of pregnant women are increased.

Riboflavin deficiency manifestations (glossitis, angular stomatitis, cheilosis, corneal vascularization, etc.) chiefly appeared during the last trimester of pregnancy and generally regressed after delivery. They were particularly frequent in poorer patients who were most markedly affected by food restrictions.

The purpose of the present study was to investigate the amount of riboflavin excreted in the urine in relation to the month of pregnancy and lactation, and to study the relation between riboflavin excretion in the urine and the appearance of clinical manifestations of ariboflavinosis. The excretion of riboflavin in the urine in normal nonpregnant individuals is said to be related to the amount of riboflavin in the diet.[6]

Our material is particularly favorable for such a study, since the women attending our prenatal care clinics are under close medical supervision, live in separate quarters of the town, and are distinguished from one another with respect to their origin and social and economic conditions. Some of the communities from which they stem even retain their own specific dietary customs.

MATERIAL AND METHODS

One hundred sixty-two pregnant women (sixty-seven primiparas and ninety-five multiparas) between 16 and 42 years of age were kept under observation from the third month of pregnancy until two months after delivery. During the same period fifty nonpregnant individuals constituting the control series were also studied. Clinical examinations, as well as determinations of the riboflavin content of fasting morning urine, were made twice a month in each of these patients by the method of Snell and Strong.[7]

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The patients studied, belonging to different social and economic strata, regularly attended the prenatal care centers of the Hadassah Medical Organization. Those of the oriental communities (Kurdish, Persians, Yemenites, etc.) were generally poor, and their diet consisted essentially of carbohydrates and was particularly deficient in proteins. The riboflavin content of their diet was therefore also very low. The average, less than 1.3 mg. daily, was relatively constant, and was far below the 2.5 to 3 mg. daily recommended for pregnant and lactating women by the Food and Nutrition Board of the National Research Council, U. S. A.[8]

The nutritional study of relatively well-to-do pregnant women revealed quite a different picture. Their riboflavin intake corresponded to or even exceeded that recommended for pregnancy. In this group the riboflavin intake varied considerably according to individual dietary habits and to market facilities. In order to maintain an approximately constant riboflavin intake, we were obliged to prescribe a constant diet containing 3.5 to 4 mg. riboflavin daily for these patients.

From among the 162 pregnant patients studied, we selected forty-six in whom riboflavin intake was *3.5 to 4 mg. daily*, and fifty-one patients with a daily intake of *0.7 to 1.3 mg.* These ninety-seven pregnant women were closely supervised during pregnancy and the two months of lactation following delivery, with a view to studying variations in riboflavin excretion throughout this period.

Unfortunately, a certain number of these patients had to be eliminated from this study because of insufficient data or unreliability concerning their riboflavin intake, or due to various pathologic conditions complicating pregnancy and lactation.

In the end sufficient data were available for only twenty-nine pregnant women of the group with low riboflavin intake (group A: 0.7 to 1.3 mg. riboflavin) and for eighteen with high riboflavin intake (group B: 3.5 to 4 mg. riboflavin). Thus, only forty-seven patients from the initial group of 162 pregnant women correspond to this study (Table I).

In order to compare riboflavin excretion in pregnancy urine with that of nonpregnant women, we examined two control groups of twenty-five each. Close relatives of the pregnant women in our study, living under identical conditions and receiving identical diets, were selected for this purpose. After the exclusion of unreliable subjects and of cases in which interference with adequate utilization of riboflavin occurred, ten subjects (group C) serving as controls for group A and eight subjects (group D) serving as controls for group B were studied (Table I).

RESULTS

Riboflavin Excretion in Nonpregnant Subjects.—In both control groups of nonpregnant subjects (groups C and D), riboflavin excretion was found to be quite constant throughout the period of study, and could be related to the riboflavin content of the diet. In group C, with an intake of 0.7 to 1.3 mg., the riboflavin urinary excretion averaged 113 γ per liter, standard deviation 66.5, with extreme values of 50 to 550 per liter (Table II).

In group D, in which the riboflavin intake was 3.5 to 4 mg. daily, the average riboflavin excretion was 466.4 γ, standard deviation 202.0, with extreme values of 100 to 1,000 γ per liter (Table II).

TABLE I. DAILY RIBOFLAVIN INTAKE IN THE FOUR GROUPS STUDIED

GROUP	PREGNANCY	NUMBER OF CASES	RIBOFLAVIN DAILY INTAKE
A	+	29	0.7 mg.–1.3 mg.
B	+	18	3.5 mg.–4.0 mg.
C	–	10	0.7 mg.–1.3 mg.
D	–	8	3.5 mg.–4.0 mg.

TABLE II. URINARY EXCRETION OF RIBOFLAVIN IN THE TWO GROUPS (C AND D) OF NONPREGNANT WOMEN

GROUP	NUMBER OF CASES	NUMBER OF URINARY EXAMINATIONS	RIBOFLAVIN DAILY INTAKE	AVERAGE RIBOFLAVIN EXCRETION γ/LITER	EXTREME VALUES	STANDARD DEVIATION
C	10	149	0.7 mg.–1.3 mg.	113	50–550	66.5
D	8	120	3.5 mg.–4.0 mg.	466.4	100–1000	202.0

The standard error of difference between the two means C and D = 19.2. The difference between the two means C and D is significant, as it is nearly nineteen times the value of the standard error of the difference.

Clinical manifestations of riboflavin deficiency were not observed in the control subjects studied, not even in those whose riboflavin excretion was strikingly low.

Riboflavin Excretion During Pregnancy and the First Two Months of Lactation.—It was found that riboflavin excretion in both groups: A (riboflavin intake 0.7 to 1.3 mg.) and B (riboflavin intake 3.5 to 4 mg.) of pregnant women considered together was lower than that of corresponding groups of nonpregnant women with the same riboflavin intake (Table III). It averaged 203.3 γ, standard deviation 152.5, for groups A and B, with extreme values of 40 to 800 γ per liter; and for groups C and D 270.6 γ, standard deviation 227, with extreme values of 50 to 1,000 per liter. However, when groups A and B were studied separately, the average riboflavin excretion in group A was almost identical with that in its control group C, and equalled 113.1 and 113, respectively (Table III).

In group A only slight variations were observed during the course of pregnancy. However, the average excretion of the last trimester was significantly lower than that in the four preceding months, the average excretion of riboflavin in the urine in the last trimester in this group (A) being *93.0 γ per liter*,

TABLE III. COMPARISON OF RIBOFLAVIN URINARY EXCRETION BETWEEN THE PREGNANT AND NONPREGNANT WOMEN

	GROUP	NUMBER OF CASES	NUMBER OF URINARY EXAMINATIONS	RIBOFLAVIN DAILY INTAKE	AVERAGE OF RIBOFLAVIN EXCRETION γ/LITER	STANDARD DEVIATION
I	A + B Pregnancy III–IX M	47	581	0.7 mg.–4.0 mg.	203.3	151.5
II	C + D	18	269	0.7 mg.–4.0 mg.	270.6	227.0
III	A Pregnancy III–IX M	29	362	0.7 mg.–1.3 mg.	113.1	55.0
IV	B Pregnancy III–IX M	18	219	3.5 mg.–4.0 mg.	352.4	145.1
V	C	10	149	0.7 mg.–1.3 mg.	113.0	66.5
VI	D	8	120	3.5 mg.–4.0 mg.	466.4	202.0

The standard error of difference between the two means IV (B pregnancy) and VI (D) = 20.7. The difference between these two means is significant, as it is nearly six times the value of the standard error of difference. The average for III (A pregnancy) and V (C) is identical.

The standard error of difference between the two means I (A + B pregnancy) and (C + D) = 15.2. The difference between these two means is significant as it is more than four times the value of the standard error of the difference.

standard deviation 21.5, in comparison with 125.8 per liter, standard deviation 64.9, which was the average excretion for the preceding four months (III, IV, V, VI) (Table IV). Moreover, in most patients of this group riboflavin excretion rose sharply during the first week following delivery (average 199.2 per liter in this first week after delivery, as compared with the last trimester 93 γ per liter) (Table IV), in spite of the fact that the riboflavin intake remained unchanged during this period. But the riboflavin excretion diminished again in the course of the first month post partum, and remained low till the end of the second month, when the study was terminated, and averaged 145.8 γ per liter for this early lactation period (Table IV). In five cases the riboflavin urinary level failed to decrease during the lactation period; and it is noteworthy that in these patients milk secretion was remarkably poor.

In group B a distinct average decrease in riboflavin excretion was observed during the last trimester. In the first week of lactation a rise in the riboflavin excretion occurred (423.6), and a considerable decrease was again observed in the course of the first and second months after delivery when lactation was well established (average 273.3 per liter) (Table IV).

Manifestations of Ariboflavinosis Related to Variations in Ariboflavin Excretion in the Urine During the Course of Pregnancy and Lactation.— None of the twenty-nine pregnant women with low riboflavin excretion in the urine showed distinct clinical manifestations of ariboflavinosis when first examined and selected for this study. In nineteen (65 per cent), clinical signs of ariboflavinosis such as glossitis, cheilosis, angular stomatitis and

TABLE IV. AVERAGE RIBOFLAVIN EXCRETION IN DIFFERENT PERIODS OF PREGNANCY AND LACTATION

GROUP	NO. OF CASES	NO. OF URINARY EXAMINATIONS	RIBOFLAVIN INTAKE DAILY	AVERAGE EXCRETION IN PREGNANCY III-VI½ MO.	STANDARD DEVIATION	AVERAGE EXCRETION IN PREGNANCY VII-IX MO.	STANDARD DEVIATION	AVERAGE EXCRETION IN PREGNANCY III-IX MO.	STANDARD DEVIATION	AVERAGE EXCRETION IN 1ST WEEK POST PARTUM	AVERAGE EXCRETION IN LACTATION I, II MO. POST PARTUM	STANDARD DEVIATION
A	29	448	0.7 mg.–1.3 mg.	125.8	64.9	93.0	21.5	113.1	55.0	199.2	145.8	71.7
B	18	271	3.5 mg.–1.3 mg.	419.3	135.0	242.8	79.7	353.4	145.1	423.6	277.3	77.2

Group A—The standard error of difference between the two means I and II = 4.72. The difference between these two means is significant, as it is more than six times the value of the standard error of the difference. The standard error of difference between the two means II and V = 8.66. The difference between these two means is significant as it is more than five times the value of the standard error of the difference. The standard error of difference between the two means I and V = 10.4. The difference between these two means is not significant, as it is less than twice the value of the standard error of the difference.

Group B—The standard error of difference between the two means I and II = 14.5. The difference between these two means is significant, as it is more than twelve times the value of the standard value of the difference. The standard error of difference between the two means II and V = 15.8. The difference between these two means is significant as it is more than two times the value of the standard error of the difference. The standard error of difference between the two means I and V = 17.6. The difference between these two means is significant as it is more than eight times the value of the standard error of the difference.

The standard error of difference between the two means III (gr. A) and III (gr. B) = 10. The difference between these two means is significant as it is nearly twenty-four times the value of the standard error of the difference.

corneal vascularization appeared during the course of pregnancy and lactation. Two patients out of the 19 developed glossitis during the fifth month of pregnancy, and this condition persisted during the entire course of pregnancy and lactation. In the third trimester of pregnancy a strikingly frequent appearance of riboflavin deficiency manifestations was noted in sixteen cases (55 per cent) (Table V). These patients presented a relatively severe picture of riboflavin deficiency, manifested by atrophic glossitis or papillitis. Angular stomatitis with the frequent occurrence of large macerated areas, seborrheic areas of the labionasal folds, cheilosis, were all observed. The patients frequently complained of severe heartburn, glosso-pyrosis, or sensation of burning in the oral structures when eating spicy food. A striking improvement in all these manifestations was observed after delivery. The subjective complaints receded in all cases in the course of the first two weeks after delivery, while the anatomic changes in the buccal mucosa improved gradually during the two months of lactation in thirteen of sixteen cases. Only one patient developed symptoms of ariboflavinosis during the lactation period (Table V). None of the ten nonpregnant controls on the same dietary regime developed deficiency symptoms during the course of this study.

In none of the eighteen patients of group B (high riboflavin intake) were any symptoms of ariboflavinosis observed throughout the entire course of pregnancy and the two months of lactation (Table V).

TABLE V. APPEARANCE OF CLINICAL SIGNS OF RIBOFLAVIN DEFIENCY IN RELATION TO THE DIFFERENT PERIODS OF PREGNANCY AND LACTATION

GROUP	NUMBER CASES	PREGNANCY III, IV, V, VI M		PREGNANCY VII, VIII, IX M		LACTATION I, II M		NUMBER OF DEFICIENCY CASES	ALL CASES
		ONSET OF DEFICIENCY SIGNS	NO DEFICIENCY SIGNS	ONSET OF DEFICIENCY SIGNS	NO DEFICIENCY SIGNS	ONSET OF DEFICIENCY SIGNS	NO DEFICIENCY SIGNS		
A	29	2	27	16	13	1	28	19 (65%)	29
B	18	–	18	–	18	–	18	–	18

DISCUSSION

All the above findings may be attributed to the additional riboflavin requirement of the growing fetus. The increasing need for riboflavin with advancing pregnancy manifested itself in well-to-do pregnant women only by the fall of riboflavin excretion in the urine, while in pregnant women with low riboflavin intake it was also apparent in the appearance of clinical symptoms of ariboflavinosis. The question may arise why pregnant women on low riboflavin intake frequently developed symptoms of ariboflavinosis, while our observations on control subjects under the same dietary conditions, as well as observations made by other authors,[9] indicate that nonpregnant volunteers kept on extremely low riboflavin intake failed to develop manifestations of ariboflavinosis. This may possibly be explained by the observation[10] that below a certain minimal level (0.35 to 0.8 mg. daily) of riboflavin intake a progressive depletion of the tissue stores of this substance occurs. It therefore appears

likely that in pregnant women on the low riboflavin intake (0.7 to 1.3 mg.) riboflavin reserves in the body are mobilized, especially during the last trimester, and clinical manifestations are due to the depletion of this vitamin from the tissues.

It may also be asked why the average riboflavin excretion in the urine was equally low in pregnant women with low riboflavin intake and in their nonpregnant controls (group A—113.1; group C—113), although additional amounts of riboflavin are needed by the growing fetus. This may be explained by the assumption that riboflavin in these pregnant women (group A) is mobilized from the tissue reserves, and riboflavin urinary excretion is therefore maintained at a relatively constant level, while deficiency symptoms follow the depletion of riboflavin.

The results obtained in this study demonstrate also that the riboflavin requirement is increased during lactation. The need for riboflavin in lactation seems, however, to be distinctly lower than that in pregnancy. The riboflavin requirement appears to increase progressively in the course of lactation, since the excretion of riboflavin decreased considerably when lactation was well established. This observation is also proved by the finding that no or little decrease in riboflavin excretion was noted in cases of agalactia and hypogalactia.

SUMMARY

A clinical study and observations on riboflavin excretion in two groups of pregnant women (group A, twenty-nine patients with 0.7 to 1.3 mg. daily riboflavin intake; and group B, eighteen patients with 3.5 to 4 mg. daily riboflavin intake) revealed an increase in riboflavin requirement in pregnancy and lactation as demonstrated by the following findings: (a) Riboflavin excretion in pregnant women with sufficient riboflavin intake (group B) was distinctly lower than that of nonpregnant individuals (group D) on the same dietary regime. (b) The decrease in riboflavin urinary levels during the last trimester of pregnancy was evident in both groups (A and B) of pregnant women studied. (c) Clinical manifestations of riboflavin deficiency occurred very frequently in pregnant women with low riboflavin intake, while they did not appear in nonpregnant control women on the same low riboflavin diet. (d) Riboflavin urinary levels rose significantly during the first week after delivery while the diet remained unchanged with respect to riboflavin content. (e) Riboflavin excretion in lactating women with sufficient riboflavin intake (group B) was distinctly lower than that of nonpregnant control subjects under the same dietary conditions. (f) A decrease in riboflavin excretion during the first two months of lactation after an initial rise during the first week following delivery. (g) The average riboflavin excretion during lactation was higher in both groups A and B when compared with that during pregnancy, especially during the last trimester (Table IV).

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A METHOD FOR THE COLLECTION AND QUANTITATIVE DETERMINATION OF SEBUM: ITS APPLICATION TO AN INVESTIGATION OF HUMAN SEBUM SECRETION

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THAT there is relatively little known about the physiology of the sebaceous glands in the human being is well attested to by the brevity of reference to this topic in most, if not all, of the modern textbooks of human physiology, and by a review of the scant medical literature available on this subject.

The apparent explanation for this dearth of information resides in the fact that the methods previously used for the study of sebum have been so laborious or complicated as to discourage sustained effort in this field, as well as being generally impractical for the purposes of clinical investigation.

In the past, three basic methods for the quantitative study of sebaceous secretion have been employed by different investigators. In 1906, Rosenfeld obtained rough estimates of sebum secretion by extracting and then weighing the amount of fat absorbed by woolen clothing worn by subjects on experimental diets for specified periods of time.[18] This same method was also used by Kuznitzky in 1913,[12] but refined in that the clothing was made fat-free prior to starting experimental observations. A second method was that in which a fat absorbent material (ether-soaked cotton, or fat-free filter paper, etc.) was applied to a limited area of skin for a given period of time. The difference between the pre- and postexperimental weights was taken as a measure of the quantity of sebum secreted, or the absorbed sebum was extracted and then quantitatively measured by one of a number of techniques. The majority of investigators[6, 7, 13, 16, 17, 19, 20] in this field have used this basic approach. A third method was introduced by Emanuel in 1936.[8] He devised a unique collecting cup which could be applied directly to the skin surface by suction. The cup contained 5 c.c. of ether as the fat-solvent medium, which was delivered to 2 square centimeters of skin area for one minute. The quantity of sebum dissolved in the ether under these standard conditions was then determined by the "Bing-Heckscher nephelometrical method." Essentially the same technique for sebum collection and determination has also been employed by Butcher and his co-workers in their investigations.[3-5]

The recent advent of a new method for analysis of skin fat, developed independently by Berry[2] and Jones,[11] has provided a simple and reliable means for the quantitative determination of small amounts of sebum.

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By using a modification of this technique, with the addition of a convenient and clinically applicable method for the collection of sebum specimens, it was possible to investigate some of the physiologic factors concerned with surface sebum in the human being, as well as alterations in facial sebum secretion associated with various emotional states in patients with acne vulgaris.[14, 15, 21]

The purpose of the present communication is to describe in detail the method for the collection and quantitative determination of sebum specimens used in this investigation, and to report some of the observations obtained by its use.

METHOD AND MATERIALS

1. *Preparation of the Skin Surface.*—The skin surface to be used as the source of sebum was prepared routinely by firmly wiping approximately one square inch of skin area, two or three times, with a cotton pledget* soaked in ether. A sebum specimen could then be obtained, at any desired time thereafter, by merely pressing the end of a glass rod to the approximate center of this area.

On all serial studies care was taken to use, as nearly as possible, the identical point within the cleansed area, from which previous sebum collections had been made.

2. *Collection of Sebum Specimens.*—It was found possible and practical to collect uniform sebum specimens, from properly prepared human skin, by the application of identical small glass rods to the skin surface, under standard conditions of pressure and time. The glass rods used were 60 mm. in length and 5 mm. in diameter. The ends of these rods had been subjected to brief grinding by an emery wheel which resulted in a uniform degree of flatness and "frostiness" of their surfaces, not obtainable in the initial process of cutting the rods to length. The grinding process, furthermore, eliminated cutting edges and imperfections which might have inflicted minor lacerations when the rods were applied under pressure to the skin surface.

A von Frey algesiometer (Fig. 1), which was adapted for use as a rod holder, provided for graded and measurable pressure of application of the glass rods to the skin surface. This device consisted essentially of a piston shaft, one end of which was equipped with a metal sleeve. The latter was of such size as to hold a glass rod firmly in place prior to and after collection of a sebum specimen. The other end of the shaft was secured to a coiled steel spring, fitted into the hollow handle of the algesiometer. A readable scale was etched into the exposed portion of the piston shaft. Pressure of application was measured by the extent to which the handle descended the length of the shaft and the scale. A point was selected which fell at approximately the midpoint of the scale and was equivalent to 400 Gm. weight. Since this application pressure was not uncomfortable, it was used routinely in all experimental procedures. The standard duration of application of the glass rods to the skin was five seconds.

The glass rods routinely were immersed in an alcohol-ether mixture, for a few seconds, a minute or two before their application to the skin. A clean and fat-free state of the rod ends was thus assured prior to contact with the surface sebum.

A single glass rod could, of course, only be used for the collection of one sebum specimen at a time. Simultaneous and serial collections were obtained by using additional (but identical) glass rods—as many as necessary—under the standard conditions noted above.

It was not found necessary to do the quantitative determination of the sebum specimens as soon as they were collected. This added much to the practicality and applicability of this method to clinical investigation. Our experience has been that up to two hours may elapse between the time a sebum specimen is collected and its determination completed, without any measurable loss of sebum having occurred in the interim.

*Fat-free, except for 0.2 to 0.3 per cent ether extractable material.

Small wooden blocks having six properly sized wells, evenly spaced, centrally located and suitably labeled, were used to keep the glass rods upright and oriented (Fig. 2). This apparatus helped prevent physical contact of the exposed ends of the rods with anything but the atmosphere, whether the rods were stationary or being transported.

3. *Quantitative Determination of Sebum Specimens.*—In 1950 Jones described a method for the quantitative assay of fatty materials (including sebum) in which the area of spread of the fat as a film, in a monomolecular layer of oil (having a tension of 20 ± 2 dynes per sq. cm.) was found to have a "sensitivity and accuracy beyond other methods" in measuring the amount of fat delivered to the oil surface.[11]

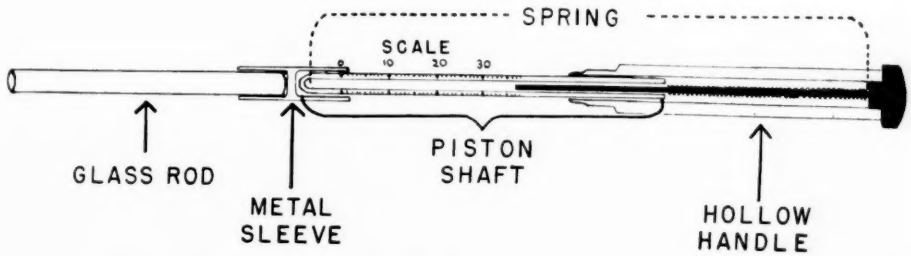

Fig. 1.—Sketch of modified von Frey algesiometer adapted for use as a rod holder and providing for standardized pressure of glass rods to skin surface.

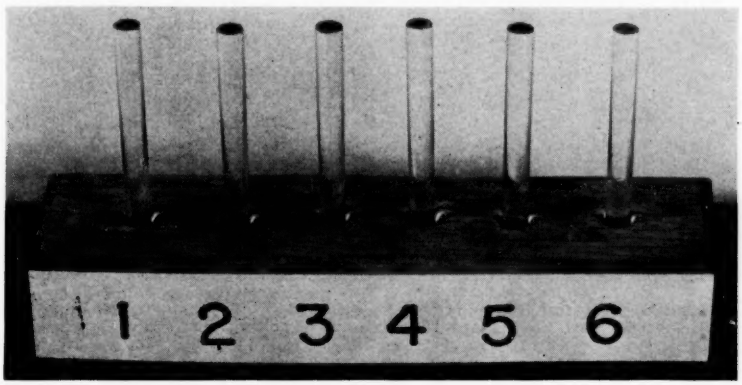

Fig. 2.—Wooden block used for keeping sebum-tipped glass rods upright and oriented.

The appartus and modified technique used in the present study consisted essentially of the following:

A large (150 by 20 mm.) paraffin-coated Petri dish was kept in a glass tray, the edges of which extended approximately ¼ inch above the Petri dish. The tray rested on a small levelling table (Fig. 3).

The Petri dish was kept filled with distilled water to a point just short of overflowing. Prior to each sebum determination the water was cleansed by passing a glass slide over its surface two or three times. When the currents produced by this maneuver had ceased (one to two minutes), a drop or two of properly prepared (heated at "low" hot plate temperature for forty-eight hours) Cenco Hyvac oil was delivered from a Tuberculin syringe, equipped with a 25 gauge needle, to the approximate center of the water surface. The oil immediately spread in a film, at first multicolored and finally greenish in color, until practically the entire surface of the water was covered with a monomolecular layer of oil.

The technique for the quantitative analysis of a sebum sample was as follows: The sebum-tipped glass rod was removed from its resting place in one of the previously described wooden blocks, care being exercised not to handle it anywhere beyond the midpoint of the rod. The sebum-coated end of the rod was then carefully touched to the oil film surface. In the presence of sebum, a clear, roughly circular area promptly appeared and expanded until an equilibrium was established between the sebum film and the piston oil layer. To assure complete delivery of the sebum from the rod to the surface film, the rod tip was gently raised and lowered a few times until further spread of the sebum film ceased. At this point a glass pane was placed over the tray (clearing the oil surface of the Petri dish by approximately ¼ inch). The area of spread of the sebum was then traced onto the glass plate by using a wax pencil. To make a permanent record, the wax pencil image was retraced onto onion skin paper, and later the exact area of spread was measured in square inches by using a planimeter.

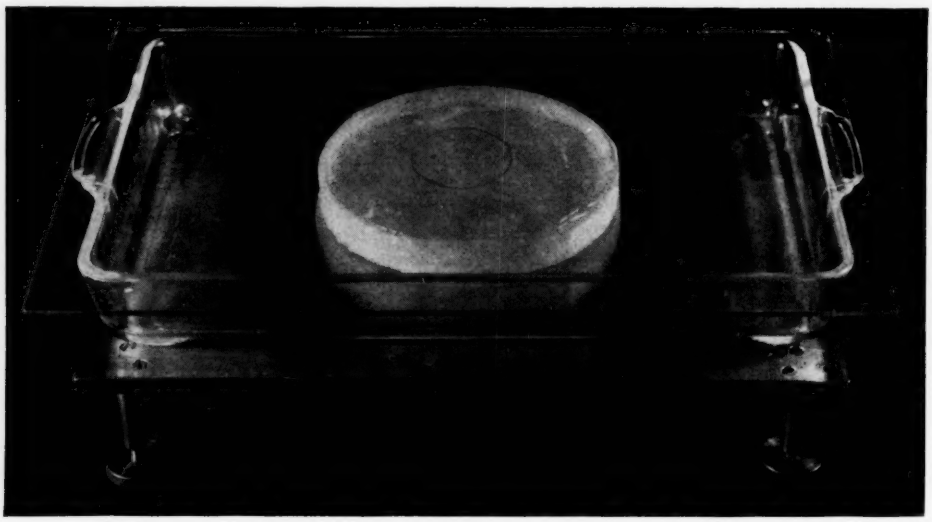

Fig. 3.—Apparatus used for the quantitative estimation of sebum specimens, consisting of paraffin-coated Petri dish, glass tray covered by glass pane and leveling table.

Comment.—The advantages of the described method over those previously used reside not only in the speed and simplicity of its performance, both as regards collection and determination, but also in its adaptability to a variety of clinical and experimental situations.

The technique of determination is sensitive and accurate.[11]

Criticism might, however, be directed at the technique employed for the collection of sebum specimens. It is not known what proportion of total surface sebum is collected by use of the glass rod method, nor is it even possible to be certain that the proportion is the same with different amounts of surface sebum. Since, however, the amount of fatty material collected increased steadily after washing, with the passage of time (see results), it seems justified to conclude that the greater the amount of surface sebum present, the greater the absolute amount collected. In addition, the consistency of the experimental results, which will be evident in the data to be presented, is strong evidence for the

belief that there is a reliable relationship between the amount of surface sebum present and the amount collected. In other words, if there were no such relationship, this method would not yield reproducible data.

Since visible sweat was present in a few of the experiments to be described the possibility that surface sweat influenced the sebum measurements had to be considered.

Sweat specimens were obtained by carefully touching a glass rod to a sweat droplet on the skin surface (forehead). These were immediately tested for spreading effect in order to avoid the factor of evaporation.

It was repeatedly found that sweat collected three and six minutes after wiping the skin with an ether-soaked cotton pledget, which removed both surface sweat and sebum, had no spreading effect whatsoever. On the other hand, it was demonstrated that sweat from an unwashed skin surface, as well as from one fifteen minutes after washing, did yield readings which were from 7 to 15 per cent of those given by sebum specimens collected under standard conditions from the same site as the sweat, and immediately after the sweat specimens were obtained. This 7 to 15 per cent spreading effect of sweat is believed to be attributable to contamination of the sweat by sebum. It is, of course, theoretically possible that even though sweat alone gives no spreading effect, the presence of sweat might result in a reading different from that obtained from the same amount of sebum alone. It is difficult to eliminate this possibility completely. However, the absence of a simple parallelism in the relation of surface sweat to sebum (sebum output *increased* in the presence of sweating in a warm environmental temperature and *decreased* in the presence of sweating during immersion of the legs in hot water—see results) suggests that such a possibility is quite remote.

On the basis of all the foregoing, it seemed legitimate to employ this method of collection in an investigation primarily aimed at the detection of alterations in the quantity of surface sebum, but not concerned with the absolute amount of sebum either in grams per unit area or unit time.

SUBJECTS

Six healthy adults, ranging in age from 25 to 35 years, voluntarily submitted to the experiments to be described, except for those involving ACTH. In the latter experiments, the subjects were three patients with migraine headache. The majority of experiments were conducted two or more times on each of four subjects. Every experimental procedure was performed on at least two individuals.

PROCEDURES AND RESULTS

1. *Removal of Facial Surface Sebum.*—By thorough washing of the face with warm water and soap, followed by a complete rinsing with cool water, or by firmly wiping an area of skin a few times with a relatively fat-free, ether-dampened cotton pledget, surface sebum could be removed. Immediate application of a glass rod to a skin surface so cleansed yielded zero readings, i.e., no spreading effect (Fig. 4).

Comment.—Since it was more simple and reliable, the ether-soaked cotton pledget technique of surface sebum removal was used in all subsequent experi-

ments. It is recognized that ether is an irritant and when repeatedly applied to the skin it may exert some influence on sebum secretion. Such a possibility does not seem to be important, however, in the short-term experimental procedures described below since the use of ether did not preclude increased, decreased or unchanged sebum output if the experimental conditions were appropriate.

2. *Reaccumulation of Facial Surface Sebum.*—Sebum collections made at one minute after removal of an hour's accumulation of facial surface sebum ("control"), and thereafter, from approximately the same site, at five-minute intervals, demonstrated that sebum reaccumulated at a rate such that approximately the prewash control level was reached within sixty minutes (Fig. 4; "right angle of nose").

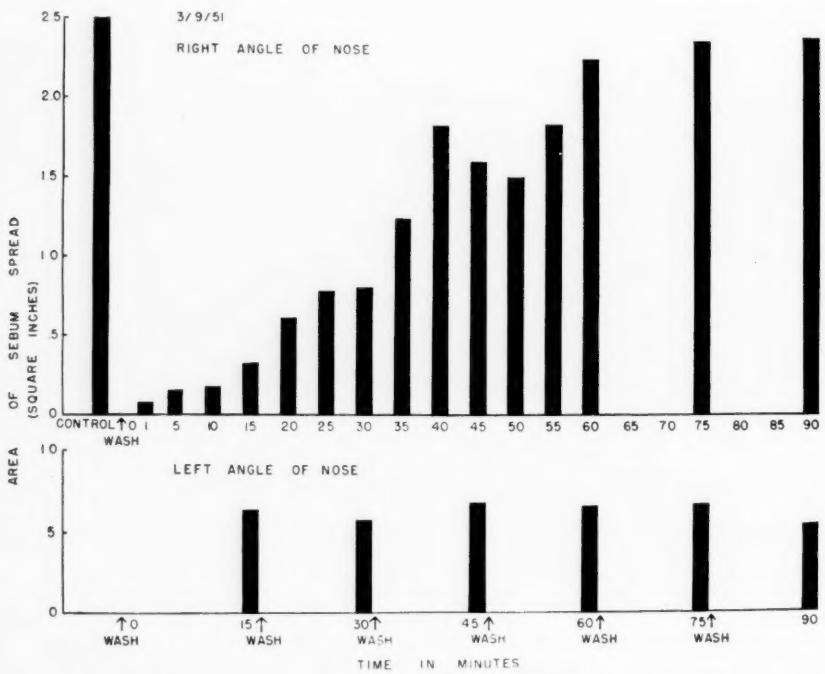

Fig. 4.—Serial sebum collections from right and left "angles of the nose" in healthy subject. The figure illustrates: (1) removal of surface sebum by washing ("Control" = sebum accumulation one hour after washing); (2) sebum reaccumulation following single washing ("right angle of nose"); (3) stability of sebum output during a tranquil period demonstrated by periodic washing at fifteen-minute intervals ("left angle of nose").

Comment.—These results generally conform to those of Emanuel[8, 9] and others,[19, 20] the only disparity being in the length of time necessary for the surface sebum to reach the previous control level. The relatively rapid rate of reaccumulation found in the present study may perhaps be explained by the fact that these sebum specimens were obtained from the "angle of the nose," a sebum-rich area.

3. *Stability of Sebum Output During a Tranquil Period.*—It was found by periodic washing of the skin of the face, and by making equally spaced sebum collections during periods of tranquillity, that practically uniform and stable sebum output in that skin

area was maintained (Fig. 4; "left angle of nose"). Periodic washing and collections at fifteen-minute intervals were continued experimentally up to a maximum of ninety minutes. The irritant effect of the ether on the skin, manifested by mild local "soreness," precluded further extension of these experiments.

Comment.—These data are opposed to those of Emanuel,[8] who observed that the more frequently surface sebum was removed the more rapidly it reaccumulated. On this basis the periodic washing in the present experiments would have yielded a steplike result instead of the stable output actually found.

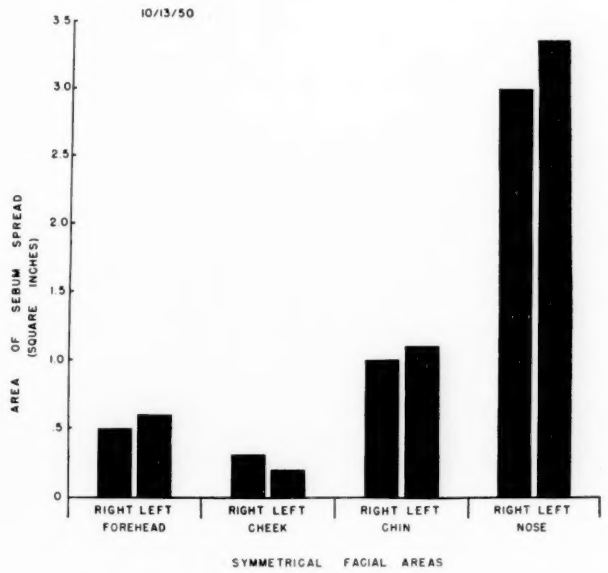

Fig. 5.—Simultaneous sebum collections from symmetrical areas of the face in healthy subject.

4. *Symmetrical Distribution of Facial Surface Sebum.*—When simultaneous sebum collections from symmetrical areas of the forehead, cheeks, chin, and nose were made, it was found that the general level of facial sebum varied a good deal from person to person, and even in the same subject from day to day. In any one individual, however, the quantities of sebum from each pair of symmetrical areas were nearly equal in amount at all times. Furthermore, in each individual, the amounts of sebum from the forehead, cheek, chin, and nose on one side of the face maintained roughly the same relationship to each other (Fig. 5).

The most sebum-rich site in every case studied was the "angle of the nose." Beyond this, the pattern of proportionality of sebum levels (from the three remaining unilateral areas) was somewhat different, and seemingly characteristic, for each individual. For example, in one subject the quantity of sebum obtained from the chin was *always* greater than that from the forehead, and the amount from the forehead was always greater than that from the cheek. In another subject the relationship was consistently forehead > chin > cheek.

Comment.—These observations support, and augment, the work of Emanuel[8] and Doupe.[6] The former previously demonstrated that there were anatomical-regional differences in sebum output with maximum levels obtained from the forehead (only head area studied) and decreasing amounts in the following

order: midchest, midback, abdomen, arms, and legs. Doupe noted that sebum secretion from anatomically symmetrical areas in normal subjects varied in amount by no more than 3 mg. per 100 sq. cm., which he considered to be insignificant.

These data also suggest that, though the facial surface sebum may vary a good deal quantitatively from time to time, this is not a localized and selective phenomenon. Rather, such fluctuations usually involve all regions of the face equally and simultaneously.

It was on the basis of these observations that the use of a *single* site for the collection of facial sebum seemed justified. Since the "angle of the nose" was demonstrated to be the most consistently sebum-rich area of the face, it was selected as the site of sebum collection to be used routinely in all experiments.

5. *Daily Variations in Facial Surface Sebum.*—Daily sebum collections were made in six healthy subjects for thirty days. The procedure adopted, in an attempt to avoid as much as possible the many extraneous factors which might conceivably influence the day-to-day level of surface sebum, included the following:

1. All subjects were instructed to avoid touching the skin of the face between the time it was washed and the time the sebum specimen was obtained.
2. Sebum collections were made at the same time every day, usually two hours after the morning washing routine.
3. All sebum collections were obtained from the "angle of the nose."
4. Note was made of daily fluctuations in activity, diet and mood of the subjects, as well as environmental factors to which the subjects were exposed.
5. A gross appraisal of the oiliness of the skin was routinely made at the time the sebum specimens were collected.

As is exemplified in Fig. 6, there were marked daily variations in the quantity of facial sebum secretion in all subjects. In the instance of Subject A (Fig. 6), the range of sebum spreading effect was from 0.69 square inch to 3.60 square inches.

Comment.—Although immediately successive daily determinations were not of the magnitude noted above, the day-to-day fluctuations in facial surface sebum secretion in all subjects were quite striking. Grossly, little change in skin oil could be detected with certainty from day to day, the only exception to this being on the days when the sebum level was at its lowest limits. Quite commonly, on these days, the subjects spontaneously commented on the fact that their "skin seems drier than usual."

On some of the days when the sebum secretion was greatest, the subjects were angry or frustrated, while on other days when the surface sebum was at its lowest ebb, they were depressed and "moody." It must be emphasized, however, that on a number of days when the sebum spreading effect was equally great or small, no apparent deviation in mood could be detected. Thus, caution is warranted regarding the significance of these observations. It would seem that, though these data are suggestive, they are in no way conclusive with respect to the significance of mood in the daily variations in facial surface sebum found in these healthy subjects.

It was not possible to make significant correlations between daily fluctuations in the output of surface sebum and alterations in environmental tempera-

ture and humidity, diet, nor with the minor changes in physical activity, which were either the consequence of the day-to-day occupational demands, or the choice, of the individual subjects.

Emanuel[8] noted that daily variations in sebum level, up to 30 per cent, occurred in normal subjects, but made no attempt to explain this phenomenon. The day-to-day fluctuations in the output of facial surface sebum found in the present study far exceed 30 per cent. This is apparent in Fig. 6, for example, where the mean daily sebum spreading effect is 1.96 square inches, and the range from 0.69 to 3.60 square inches. Some of the discrepancy between the findings of Emanuel and those in this study might be related to the difference in the methods used, to the conditions of collection, or to the choice of site for sebum specimens.

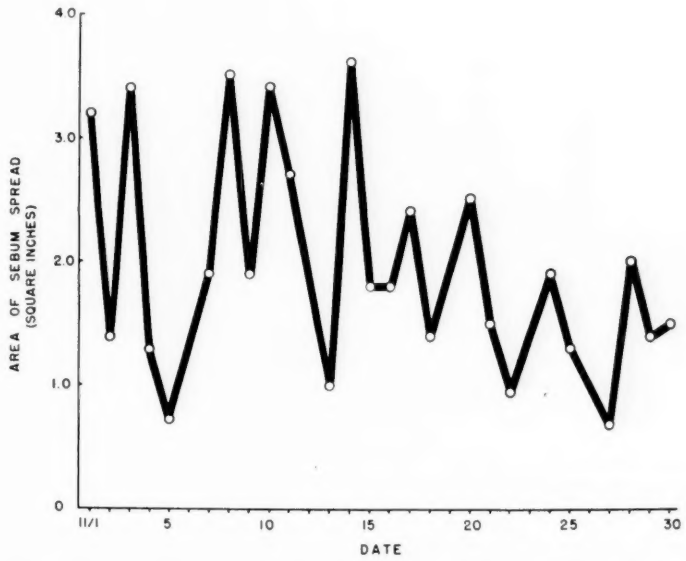

Fig. 6.—Daily sebum collections in healthy subject (subject A) over thirty-day period. Note marked fluctuations in facial sebum output from day to day.

The net results of both studies, however, are in accord as to the occurrence of relatively marked daily fluctuations in the output of surface sebum. Although an attempt was made in the present investigation to correlate these changes with a variety of possible influences, no single factor of prime importance could be found responsible. Thus it might be concluded that such alterations occur as the result of the combined effect of a variety of factors, rather than of any one of these alone.

6. *Effects of Miscellaneous Procedures on Facial Surface Sebum.*—

a. *Exercise:* Four exercise experiments were carried out on two subjects. Sebum collections were made from the "angle of the nose" at fifteen-minute intervals; two before exercise and four after. The standard exercise used was the Masters two-step procedure in

which two steps, each 9 inches in height, are ascended and descended for a period of five minutes. Prior to and after this short exercise period the subjects were inactive and relaxed. The room temperature was constant throughout the experiments.

As exemplified by Fig. 7, in each of the four experiments a delayed rise in the output of facial surface sebum occurred which was first apparent thirty-five to sixty minutes after exercising. The maximum rise was from 50 to 240 per cent of the average pre-exercise level.

b. *ACTH:* Because it has been reported that administration of ACTH is associated with acneiform eruptions,[1] four sebum experiments were performed on three patients who were receiving this agent for the purpose of evaluating its effect on their migraine headaches. Serial sebum collections were made from the "angle of the nose" at thirty-minute intervals; two before and six after the intramuscular injection of 35 to 150 mg. of ACTH. Throughout the experimental period the room temperature remained constant, and the patients were recumbent.

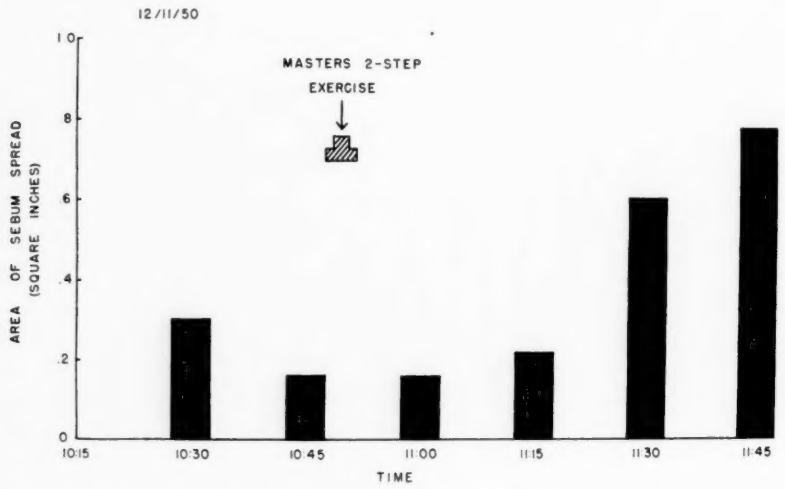

Fig. 7.—Delayed rise in facial sebum output following five-minute exercise period.

In three experiments on two patients there was a delayed rise in the surface sebum which reached its maximum (90 to 180 per cent) one and one-half to two hours after the ACTH injection. In the single experiment performed on the third patient no such rise occurred, the sebum output remaining essentially unchanged throughout the period of observation (four hours).

c. *Environmental temperature:* Since others[4, 7, 12] have reported sebum secretion to be increased in hot and decreased in cold environmental temperatures, four experiments of this type were conducted on two subjects. Serial sebum collections were made from the "angle of the nose" before, during, and after exposure of the subjects for thirty minutes to warm or cool environments. Two fifteen-minute sebum collections were made while the subjects spent an initial thirty minutes in an environment of 32° to 33° C., two more collections were taken during thirty minutes spent in a warm (40° C.) or cool (10° C.) external temperature, and, finally, two fifteen-minute collections were made after the subjects returned to the original temperature for thirty minutes. Throughout the experiments the subjects were inactive and relaxed.

As may be seen in Figs. 8 and 9, the facial sebum output increased (130 per cent and 250 per cent) during the period spent in the warm environmental temperature, and

decreased (45 per cent and 60 per cent) during the first thirty minutes spent in the cold. In all experiments the output of surface sebum came back toward, or to, the previous control level, when the subjects returned to the original environmental temperature.

d. *Hot air:* Three experiments were carried out on two subjects in which a stream of hot (40° C.) air was directed at one nasolabial fold for thirty minutes. The remainder of

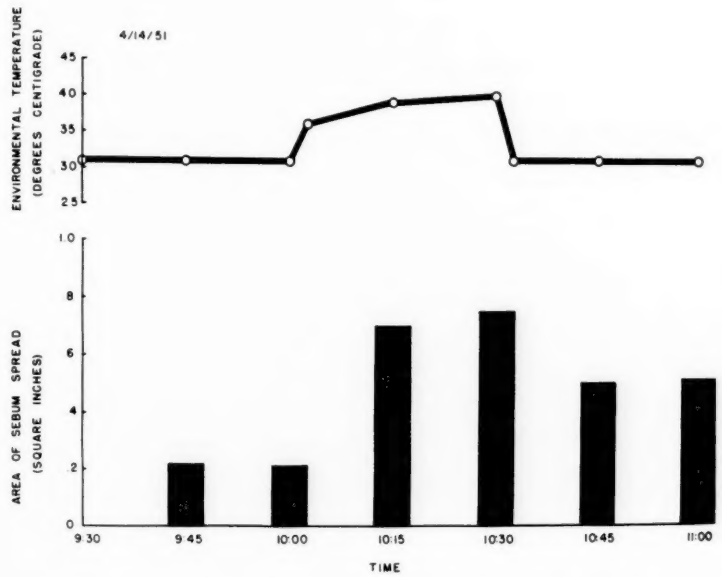

Fig. 8.—Abrupt rise in facial sebum output during thirty minutes spent in warm (40° C.) environment.

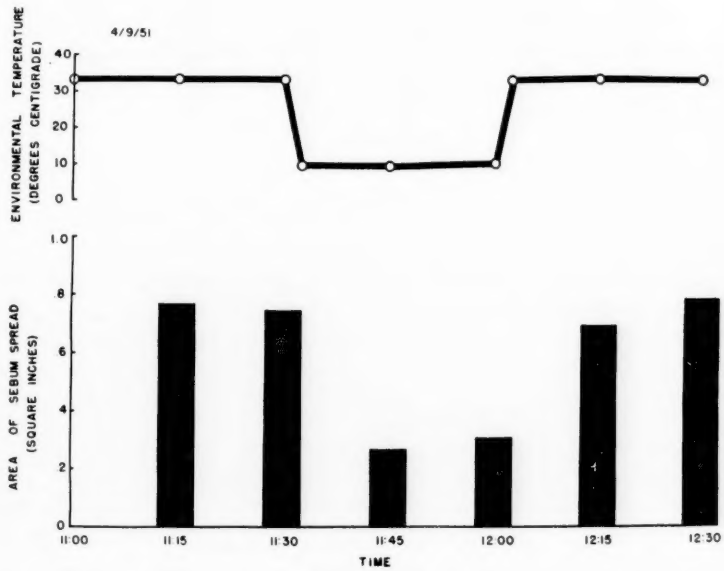

Fig. 9.—Abrupt fall in facial sebum output during thirty minutes spent in cool (10° C.) environment.

the face, and particularly the other "angle of the nose," was protected by a shield from the hot air stream. Serial sebum collections were made from *both* angles of the nose at fifteen-minute intervals, and skin temperature measurements were made over both sites (Hardy radiometer[10]) before, during, and after exposure of one nasolabial area to the hot air stream. Throughout the experiments the subjects were relaxed and the room temperature remained constant.

In each of these experiments there occurred an increase (12 per cent, 45 per cent, 129 per cent) in sebum secretion at the "angle of the nose" during the time it was exposd to the hot air stream. Simultaneously, there was no significant change in sebum output from the other "angle of the nose." Likewise, during the thirty minutes exposure to the hot air there was an increase (2° C.) in the skin temperature on the exposed side, but practically none (0.1° C.) on the unexposed side. A return of the sebum output toward the initial control level occurred on the exposed side coincident with decrease in the skin temperature on that side after stopping the hot air stream. There was no evidence of facial vasodilatation in any of these experiments.

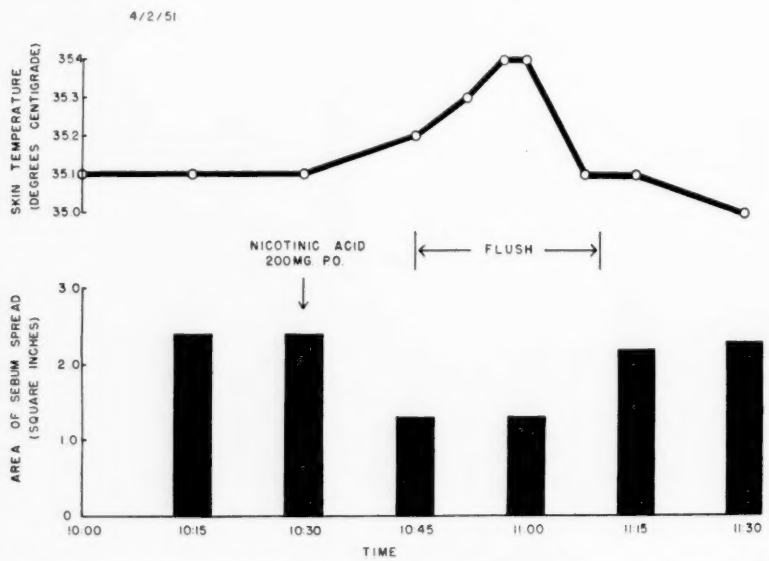

Fig. 10.—Abrupt fall in facial sebum output during period of facial vasodilatation and increase in skin temperature induced by nicotinic acid.

e. *Immersion of legs in hot water:* In two experiments on two subjects, serial sebum collections were made from the "angle of the nose" at fifteen-minute intervals before, during, and after immersion of the legs in water at a temperature of 42° to 43° C. This procedure produces a rise in skin temperature of the face and facial flushing.

In both experiments there was a decrease in facial surface sebum secretion during immersion amounting to 26 per cent of the control output in one case and to 50 per cent in the other.

f. *Nicotinic acid:* Each of two subjects was given 200 mg. of nicotinic acid by mouth for the purpose of producing a facial flush. Serial sebum collections were made from the "angle of the nose," and skin temperature measurements were made over the ipsilateral malor eminence by using a Hardy radiometer[10] before and after administration of the agent.

As shown in Fig. 10, an abrupt fall in facial surface sebum, 45 per cent in one case and 70 per cent in the other, occurred during the period of facial vasodilatation and in-

crease in skin temperature. A return of the sebum output toward the control level occurred in both cases, with subsidence of facial flushing and fall in skin temperature.

Comment.—It was initially inferred that there might be some direct relationship between vascular changes in the skin and sebum secretion, since heating the skin of the face by external means (hot environmental temperature and hot air stream) lead to increased sebum output. An obvious assumption might be that such heating of the skin resulted in facial vasodilatation and that increased sebum output was a consequence of this vascular change. Vasodilatation was not evident, however, during the hot air stream experiments, and only questionably present in the hot environmental temperature studies. In the two types of experiments designed to produce maximal facial vasodilatation (immersion of legs in hot water and nicotinic acid), there was actually a fall in sebum output, even though the skin temperature rose. Thus, the results of these exploratory experiments do not suggest any simple hypothesis about the mechanism of facial sebum secretion in terms of its possible relationship to vasomotor changes in the skin.

The similarity between the effects of ACTH and exercise on facial sebum secretion is interesting, and perhaps is the result of a common mode of action.

SUMMARY AND CONCLUSIONS

A new method for the collection and quantitative estimation of surface sebum is described which has been applied to an investigation of some factors influencing facial sebum secretion in the human being.

It was found that facial surface sebum: (a) Was removed by washing the skin with soap and water, or with an ether-soaked cotton pledget. (b) Reaccumulated at the "angle of the nose" at such a rate that approximately the pre-wash control level was reached within sixty minutes. (c) Was maintained at practically a uniform level during periods of tranquillity. (d) Had a symmetrical distribution, and variations in facial sebum secretion were not localized and selective but occurred in all areas of the face equally and simultaneously. (e) Fluctuated markedly from day to day. It was not found possible to correlate these alterations with any single factor. Thus it is suggested that this phenomenon might have been a result of the combined influence of several. (f) Was influenced by a variety of experimental procedures associated with altered temperature of the facial skin. The results of these studies did not, however, lend themselves to any simple interpretation with regard to the relationship, if any, between sebum secretion and vasomotor changes in the skin of the face.

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VARIATIONS IN THE PROTEOLYTIC AND THE ANTIPROTEOLYTIC REACTIONS OF SERUM: EFFECT OF DISEASE, TRAUMA, X-RAY, ANAPHYLACTIC SHOCK, ACTH, AND CORTISONE

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SIGNIFICANT increases in the antiproteolytic activity of serum occur in disease states, especially in those patients with malignant neoplasms, acute infections, pregnancy, and in the postoperative state.[1-7] It has been suggested that this antiproteolytic material is either a protective antienzyme or a product of protein digestion by proteolytic emzymes. If either of these explanations is correct, one would expect to find changes in the proteolytic activity of the serum correlated with changes in the level of the antiproteolytic activity.

Under normal conditions human blood plasma or serum shows little spontaneous proteolytic or fibrinolytic activity, but rather contains an inactive precursor called plasminogen.[11] Dastré[12] first indicated that proteolytic enzymes were present in the blood. As shown by Delezenne and Pozerski and others, treatment of serum with chloroform results in a definite increase of proteolytic activity.[13-15] Yamakawa[16] produced activity by the use of other substances, including alcohols. The most consistent and rapid activator of plasminogen has been streptokinase,[17, 18] but Gerheim and others have also reported the observation of an active staphylokinase,[19] the activity of which has been confirmed in this laboratory.[20]

The isolation and identification of the proteolytic and fibrinolytic enzymes of serum is still the subject of study. As early as 1936 Milstone[18] was able to partially separate the precursor plasminogen. Tagnon[15] and others partially separated the chloroform activated enzyme. At the present time attempts are being made by Christensen and associates,[21] among others, to further purify plasminogen or plasmin and isolate the proteolytic or fibrinolytic enzyme. Plasmin (activated plasminogen) is similar to trypsin in that it is a proteolytic enzyme which affects fibrin, fibrinogen, casein, gelatin, hydrolysin, and other substrates, but they are not identical because of a difference in the optimum pH for activity, quantitative differences in the breakdown of the above substrates, and the results of tests on specific substrates.[17, 22]

A method for assay of this proteolytic precursor (plasminogen) in small quantities of serum has been developed along with objective methods of determining the antiproteolytic activity.[6, 23] These substances have been studied

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in several animals and in human patients under varying conditions. Sera of some of the other animals have been shown to have more spontaneous activity than the human being, while others have equal or even lesser activity.[11]

Increased fibrinolytic activity of the blood has been observed in shock,[24, 25] after injection of adrenalin,[26] at post mortem after death from severe trauma,[27] with peptone shock in dogs,[28, 29] after electrically induced convulsions,[30] in some liver diseases,[31-33] and in hemophilia.[34] Since we had previously observed an elevation in the antiproteolytic activity of the serum in postoperative patients, patients who had been subjected to trauma, and patients and animals who had been subjected to x-ray therapy, it seemed desirable to study together the proteolytic and antiproteolytic activity of the serum produced by these modalities.

MATERIAL

Blood was obtained from patients with and without disease, patients receiving x-ray therapy, nitrogen mustard, Coley's toxin, radioactive iodine, cortisone, or ACTH therapy, patients before, during, and after operation, and patients who were the victims of significant trauma. After permitting the blood samples to clot, they were centrifuged and the serum withdrawn and frozen until the analyses could be performed. In all instances where possible, a control blood sample was obtained before treatment. The time sequences varied somewhat, with the first specimen being obtained from three minutes to thirty minutes after treatment, but under ordinary circumstances samples were obtained at one, two, four, eight, twelve, and twenty-four hours after treatment or injury. In rare instances, hourly samples for twenty-four hours were obtained.

METHODS

Proteolytic activity was determined by the method previously described[23] and modified as follows: in place of the blank using the blue dye T-1824, the color produced by the reagents themselves is used as the blank in each series. Using the blank as a reference, the galvanometer index is placed at 100 per cent transmittance or 0 concentration. The per cent transmission is then read directly and the amount of tyrosine produced is determined from the graph.

Antiproteolytic activity (APL) was determined by a method previously reported.[6]

RESULTS

I. *Correlation of Plasminogen Activity With the Disease State in Patients Receiving No Special Treatments.*—

A total of 143 sera from 127 patients were tested, and an attempt was made to correlate the results of the proteolytic activity with the disease state of the patient. Definite variations among patients have been shown in the amount of plasminogen demonstrated by this test. Using streptokinase activation, which in general is the most rapid and complete, the amount of tyrosine liberated has varied from 0.071 mg. to 1.162 mg.; with protamine activation, the tyrosine liberated has varied from 0.000 to 0.395 mg. With spontaneous activation, the tyrosine liberated varied from 0.000 to 0.305 mg.

The results of these tests are reported in A, B, and C below. The streptococcal infections are treated separately in A because of the positive results obtained.

A. Streptococcal infections (17 patients): Seventeen patients were studied with plasmin activity determination and streptococcal antifibrinolysin tests.*[35] There was a direct correlation between positive antifibrinolysin tests and decreased activation with streptokinase in five tests on four patients with late streptococcal infections or recovering from streptococcal infections (Table I). Three other patients gave the typical decreased activation by streptokinase, and all had had recent acute upper respiratory infections. Only one of these three patients, No. 7 in Table I, had a streptococcal antifibrinolysin test performed which showed a normal lysis time, one week after a severe pharyngitis.

B. All diseases (127 patients): The 127 patients were arbitrarily divided into several groups: *I*, thirty-eight patients with malignant neoplasm; *II*, nine patients with tuberculosis; *III*, eight patients with streptococcal infections; *IV*, twenty-one patients with benign tumor or with no apparent disease; *V*, thirty-eight patients who had other types of disease, including heart disease, liver

TABLE I. ACTIVATOR

PATIENT	APL % NORMAL	S*	P	O	SKP AFL	
1. M. R. A96315	228	0.071	0.069	0.035	Lysis >24 hr.	(+)
2. J. S. C20147	143	0.156	0.175	0.137	Lysis >24 hr.	(+)
3. E. P. B10627	171	0.08	0.225	0.048	Lysis >24 hr.	(+)
4. J. E. C18410	157	0.15	0.187	0.045	Lysis >24 hr.	(+)
5. J. E. C18410	125	0.123	0.228	0.092	Lysis >24 hr.	(+)
6. M. D. 27379	100	0.953	0.148	0.138	Lysis 3-8 hr.	(±)
7. G. M. Employee	100	0.162	0.165	0.018	Lysis 30 min.	(−)
8. L. R. 26887	243	0.858	0.128	0.033	Lysis 30 min.	(−)
9. E. M. C7688	>214	0.336	0	0	Lysis 30 min.	(−)
10. J. H. DC1306	>286	0.338	0.06	0.025	Lysis 30 min.	(−)
11. F. F. B13991	100	0.857	0.283	0.113	Lysis 30 min.	(−)
12. A. D. 68641	114	0.452	0.21	0.15	Lysis 30 min.	(−)
13. M. C. C15481	243	0.416	0.082	0.035	Lysis 1 hr.	(−)
14. C. C. No. 1	187	0.515	0.04	0	Lysis 30 min.	(−)
15. C. C. No. 2	138	0.085	0.018	0	Lysis 1-3 hr.	(−)
16. C. C. No. 3	87	0.385	0	0	Lysis 1-3 hr.	(−)
17. M. B. B98840	>214	0.797	0.075	0.003	Lysis 30 min.	(−)

Results in milligram tyrosine produced with patients on whom streptococcal antifibrinolysin tests were done on blood drawn at same time as blood for proteolytic activity. Note diminished relative streptokinase activity in all patients without lysis and in one other (No. 7) who recently had an upper respiratory tract infection. C.C. No. 1, 2, and 3 were three children from Children's Center at New Haven.

S = streptokinase activator. P = protamine activator. O = spontaneous activation. SKP AFL = streptococcal antifibrinolysin test. APL = antiproteolytic test.

TABLE II

PROTEOLYTIC ACTIVITY	GROUPS			
	I	IV	V	II, III, IV, V, VI
Range	0.008-0.182	0.015-0.206	0.000-0.172	0.000-0.206
Mean	0.060	0.075	0.071	0.074

Range and mean of spontaneous plasmin activity as measured by milligrams tyrosine produced correlated with the patient's disease. Group I, patients with malignant tumor; Group IV, patients with no evident disease or benign tumor only; Group V, all other diseases except patients with streptococcal infection (Group III), tuberculosis (Group II), and patients who had definitive therapy for carcinoma and were without evidence of carcinoma at time of test (Group VI). The last column contains the data for the combined group of patients without evidence of malignant neoplasm (Groups II through VI). Note that there is little variation in the mean for the three groups of diseases other than malignant tumor.

*These tests were performed in the Laboratory of Pediatric Bacteriology, New Haven Hospital, by Mrs. Mildred Fusek through the courtesy of Dr. Edward Rabe.

disease, diabetes, and infections of various types; and *VI*, a group of five patients who had had definitive treatment for carcinoma without clinical evidence of recurrence. Four patients with diagnosis not definitely proved are excluded from the results.

In Table II only groups I, IV, and V are treated separately because of the very small numbers of cases in the other groups. It is apparent that the sera from patients with malignant neoplasia (Group I) in general developed slightly less proteolytic activity spontaneously. This is more evident in the mean values than in the range of activity. In addition, the ratio of patients

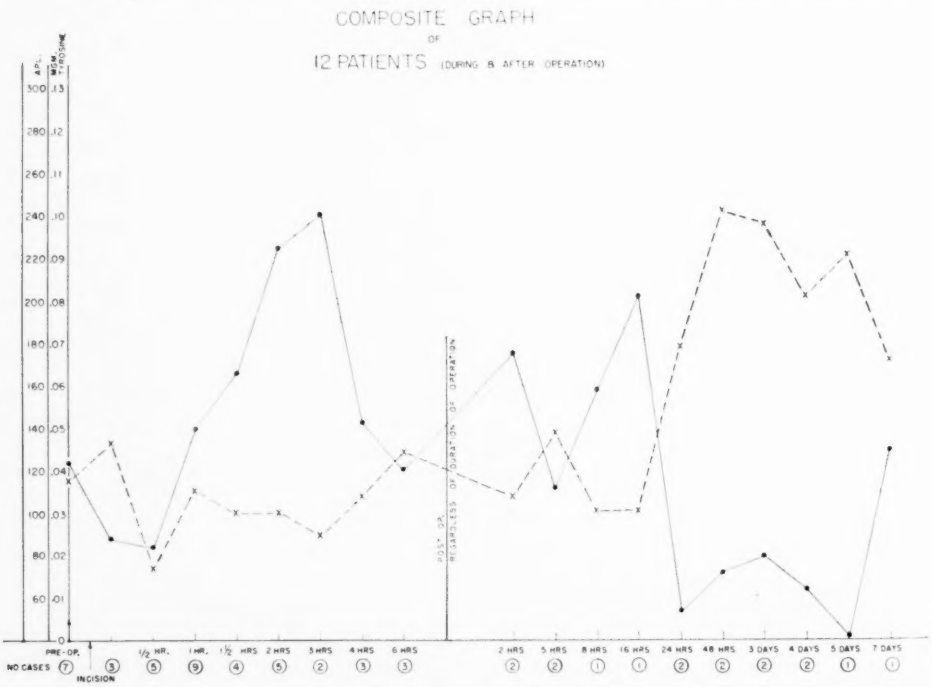

Fig. 1.—In this and all subsequent figures the spontaneous proteolytic activity in milligram tyrosine produced is indicated by the ·, while the antiproteolytic activity (APL) in per cent of a normal control is indicated by the x.

The time intervals after incision are indicated below, as are the number of patients from whom a specimen was obtained at each time.

Note the marked increase in proteolytic activity beginning one hour after the incision. The fluctuations in the first twenty-four hours after operation are undoubtedly due to the postoperative reactions of the patients. The rise in APL activity and decrease in proteolytic activity beginning twenty-four hours after operation is obvious.

whose sera developed less than half the maximum activity with each type of activation was significantly greater in the group with malignant neoplasia as opposed to all other groups.

C. Correlation of plasminogen activity with the antiproteolytic titre (12 patients): Since the sera of patients harboring malignant tumors have ordinarily high antiproteolytic titers, the relation of this to the plasminogen level was next studied. Certain consistent differences were observed (Table III). It is apparent that the sera from patients with very high antiproteolytic (APL) titers (above 200 per cent of normal) show the least plasmin activity.

while those in the group with low APL titers show the most activity. Those patients with sera in the intermediate APL ranges (125 to 149 per cent and 150 to 199 per cent of normal) fall into an intermediate group with relation to proteolytic activity.

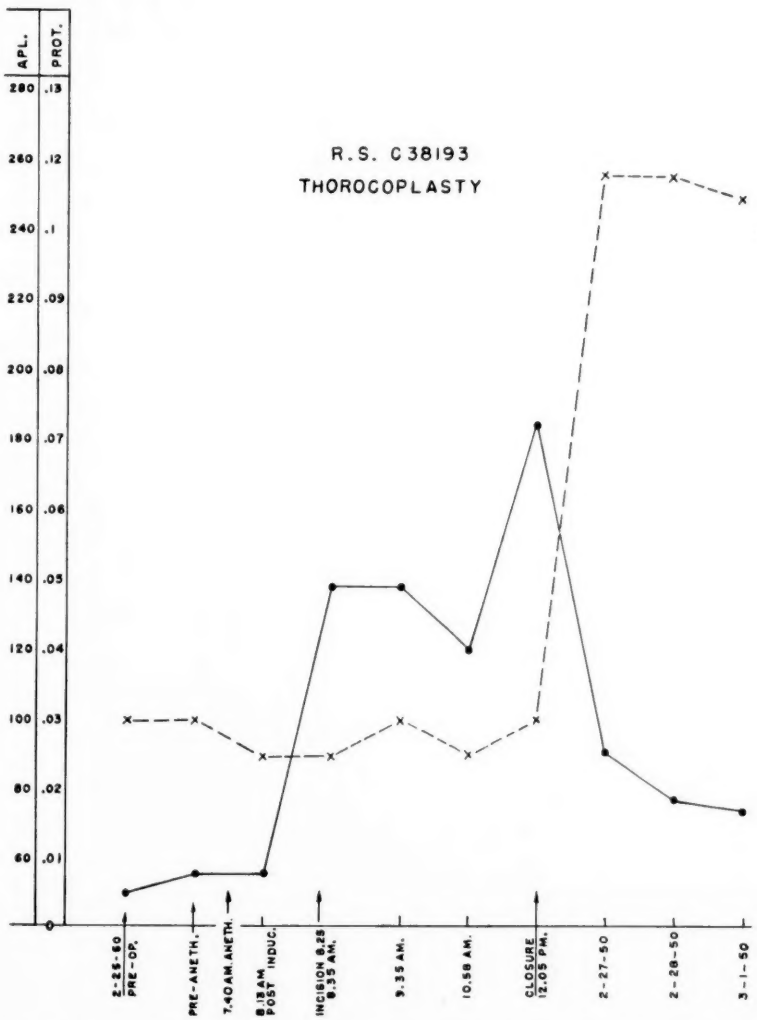

Fig. 2.—An individual subjected to a traumatic operation (thoracoplasty). Note the rise in proteolytic activity ten minutes after incision and lasting until closure with a postoperative return to normal while the APL activity rises markedly.

The obvious suggestion is that this decreased activity in the sera of patients with high APL activity is due to the presence of APL material in the plasminogen. However, our standard test for APL activity[6] failed to show any such activity in the euglobulin fraction containing the plasminogen, and indeed all activity was found to remain in the supernatant after the precipitation of the euglobulin fraction.

TABLE III

PROTEOLYTIC ACTIVITY	APL			
	200%	150%-199%	125%-149%	BELOW 125%
Range	0.000-0.138	0.000-0.270	0.000-0.230	0.000-0.305
Mean	0.049	0.056	0.068	0.080

Variation in spontaneous proteolytic activity with antiproteolytic activity. The APL activity is recorded in terms of per cent of a normal control. The inverse relationship is particularly obvious in the means.

II. Patients Subjected to Tissue Trauma, Including Therapeutic Measures.—These patients are an entirely separate group from the above 127.

A. Patients subjected to surgery (20 patients): Results of these studies are not as clear-cut as with other treatments because of the effects of the many drugs used for anesthesia, treatment of shock, repeated transfusions, and other

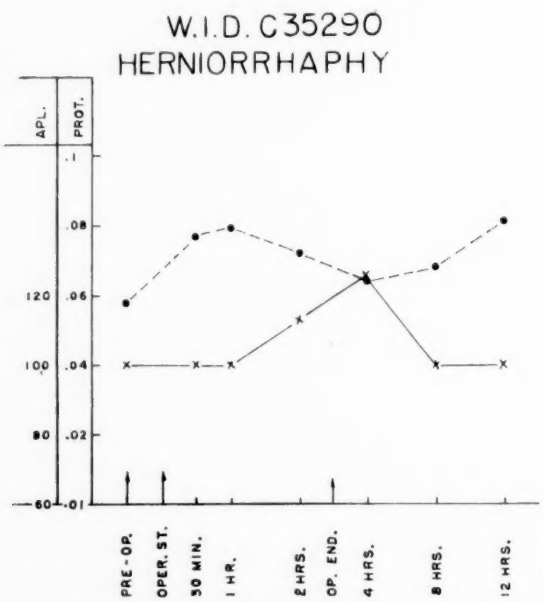

Fig. 3.—An individual subjected to a less traumatic operation. Note minimal changes in both proteolytic and APL activity. In this one chart the ·'s are connected by broken lines and x's by complete lines, the reverse of all other charts.

therapeutic agents. From Fig. 1 it will be observed that there is a slight preoperative elevation of proteolytic activity with a drop following premedication and a marked increase during the operation. This increase occurs during the major traumatic part of the procedure. At the time of closure there is a drop in proteolytic activity with a variable immediate postoperative course, and after twenty-four hours with adequate recovery of the patient a subsequent drop to preoperative levels or below. In contrast to the proteolytic activity the APL material decreases during operation, and in general shows an inverse relationship to the proteolytic activity.

With major shocking operations involving much tissue trauma, there is a markedly exaggerated reaction of this general type (Fig. 2) which can be

contrasted with the reaction to lesser operations such as herniorrhaphy or removal of a neurofibroma (Fig. 3).

The preoperative increase in proteolytic activity is possibly the result of adrenal stimulation. It has been observed that those patients fearful of the impending procedure are more likely to show a significant preoperative effect (Fig. 4). The effect of adrenal hormones is shown in subheading E (below).

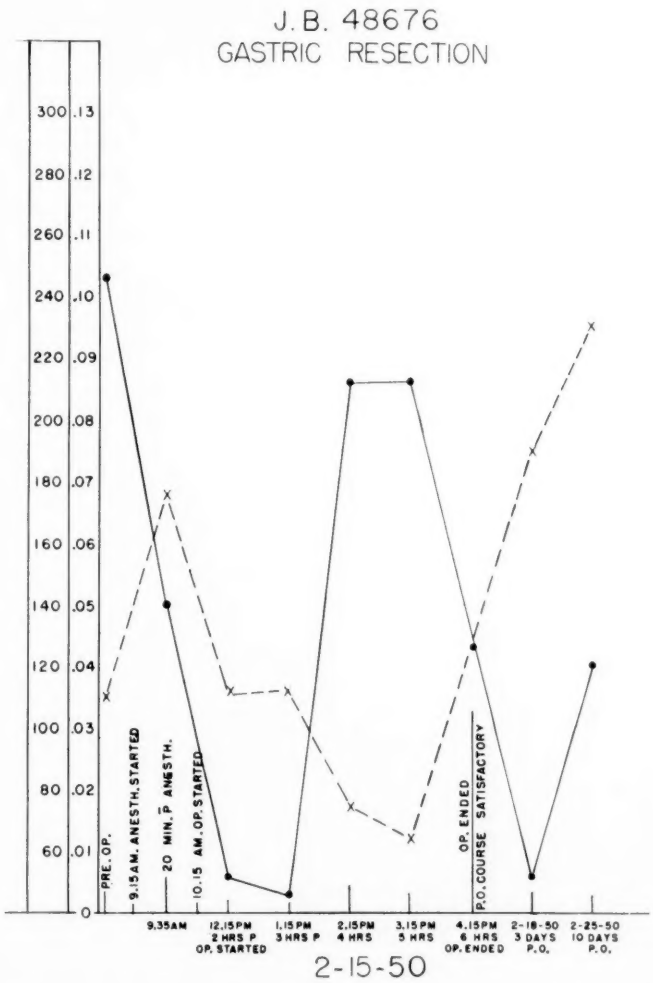

Fig. 4.—Patient who had major traumatic surgery. Note high preoperative level of proteolytic activity. This patient had had two previous operations for the same condition with severe reactions, and was obviously anxious. There is an operative rise in proteolytic activity and decrease in APL activity with a postoperative reversal.

B. Patients subjected to radiation therapy (15 patients): Patients were studied who were subjected to x-ray therapy for treatment of malignant tumor. These patients are better controlled since they received very few drugs or treatments other than x-ray during the experimental period. A definite increase

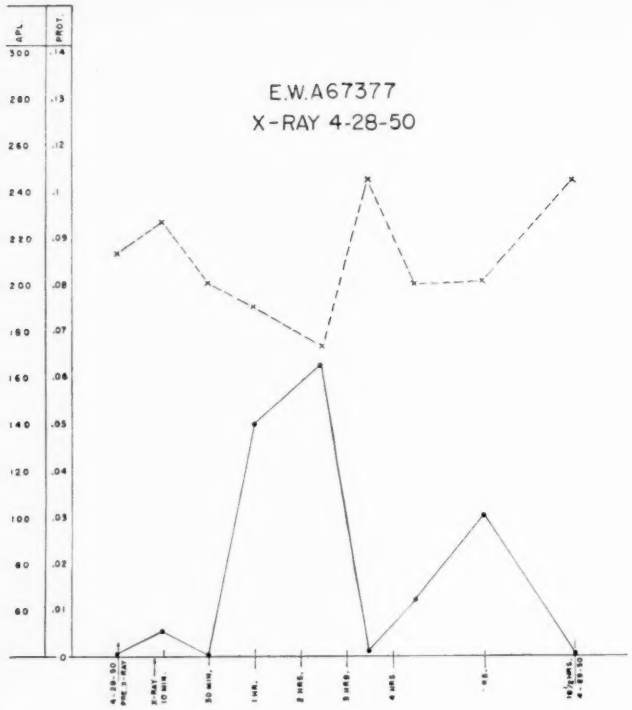

Fig. 5.

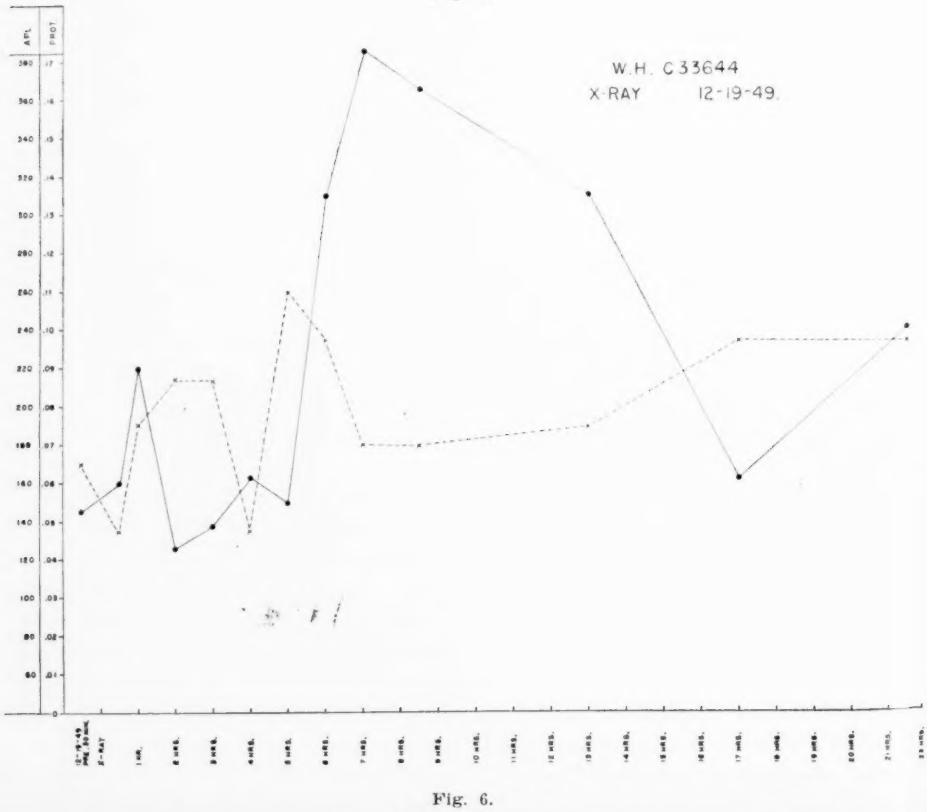

Fig. 6.

Figs. 5 and 6.—X-ray therapy patients, first treatment in each case. There is an obvious rise in proteolytic activity with a simultaneous decrease in APL activity. In most cases treated with x-ray two peaks are observed as with these particular cases, usually at about one-half to two hours and six to ten hours after treatment.

in proteolytic activity of serum occurred within sixty minutes in most instances, and once again the same inverse relationship with the APL reaction was observed (Figs. 5 and 6).

It was also observed that those patients who showed the most increase in proteolytic activity were those whose tumors showed the most response to therapy.

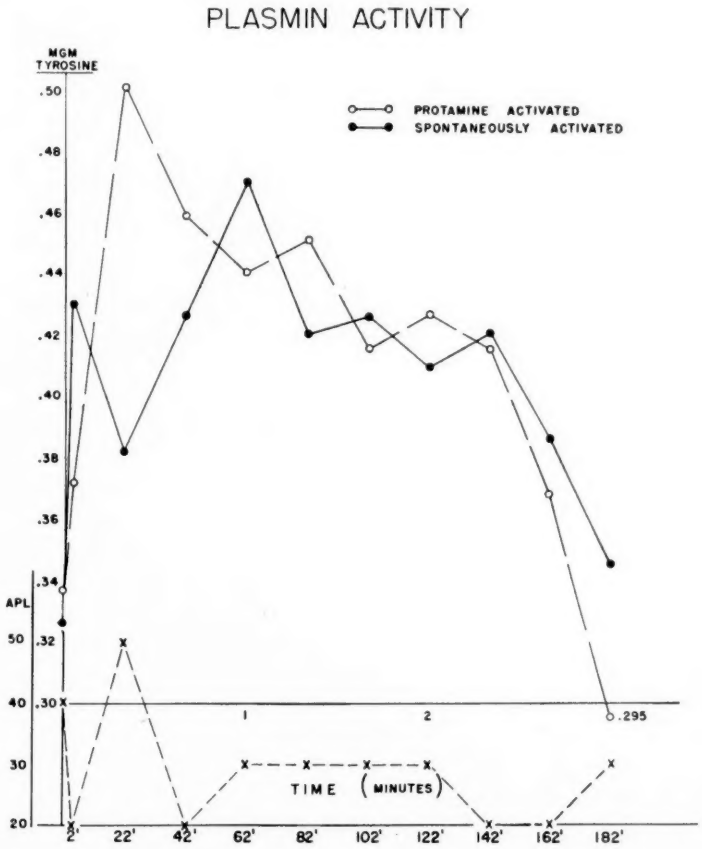

Fig. 7.—Dog subjected to anaphylactic shock. Sudden rise in proteolytic activity within two minutes after injection at a time corresponding with shock in the animal. The blood pressure began to rise at about one hundred thirty minutes and was almost normal at three hours (one hundred eight minutes). At this time the proteolytic activity had also returned to normal. The protamine activated proteolytic activity follows closely the spontaneous activity.

C. Patients receiving treatments for tumor such as nitrogen mustard, I 131, Coley's toxin (8 patients): Patients were treated with these agents and no consistent or significant variations in the reactions were observed. With nitrogen mustard therapy there were significant changes in some cases, and these again were in those patients who showed the most response to therapy.

D. Anaphylactic shock in the dog (2 dogs): Two dogs were sensitized to a human spleen extract as a part of another experiment. Anaphylactic shock

was then induced with a proper dose of the same spleen extract. There was definite increase in proteolytic activity, and here again there was a partial inverse relationship with the APL (Fig. 7).

E. Accidental trauma (4 patients): Three patients admitted with moderately severe injuries showed high spontaneous proteolytic activity and low antiproteolytic (APL) activity at the time of admission to the Emergency Room,

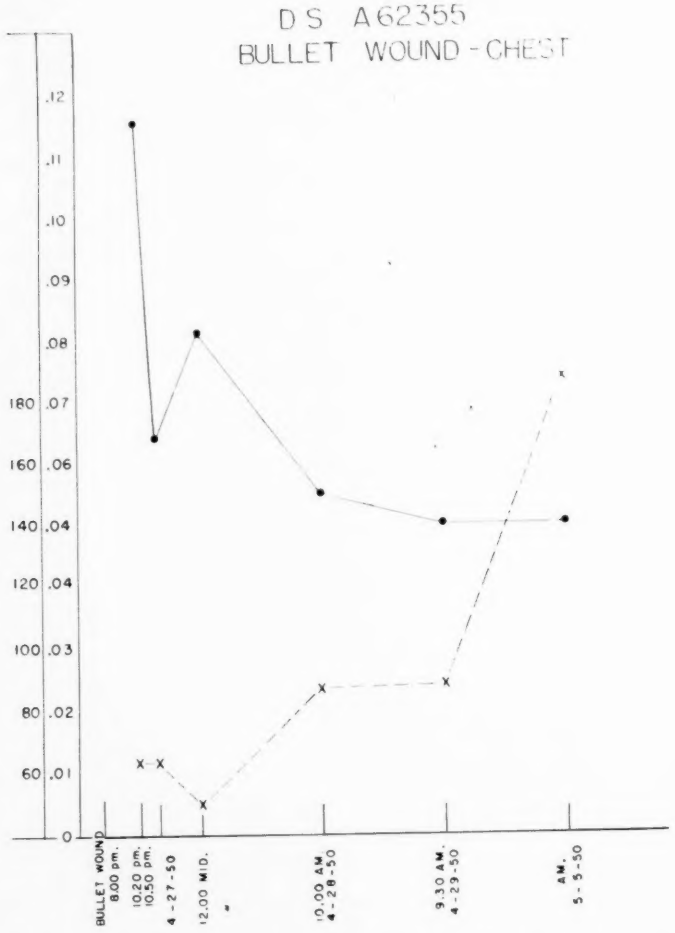

Fig. 8.—Patient wounded by 45 caliber bullet. Note steady rise in APL and fall in proteolytic activity in this patient in contrast to the following patient (Fig. 9).

followed by a rapid decrease in proteolytic activity and a slower rise in APL activity following recovery from shock (Figs. 8 and 9). One patient showed a second increase in proteolytic activity with decrease in APL activity two hours before death from recurring shock (Fig. 9) in contrast to the patient who recovered (Fig. 8).

F. Adrenal effect; ACTH and cortisone (8 patients, 6 ACTH, 2 cortisone): The most marked increase in proteolytic activity occurred in those patients treated with ACTH or cortisone, in minimal therapeutic doses (as little as 10 mg. ACTH and 100 mg. cortisone). There was in no case, however, a corresponding decrease in APL activity. Indeed, there was less change in the APL in these cases than with the lesser increases of proteolytic activity after surgery, x-ray, etc. (Figs. 10 and 11).

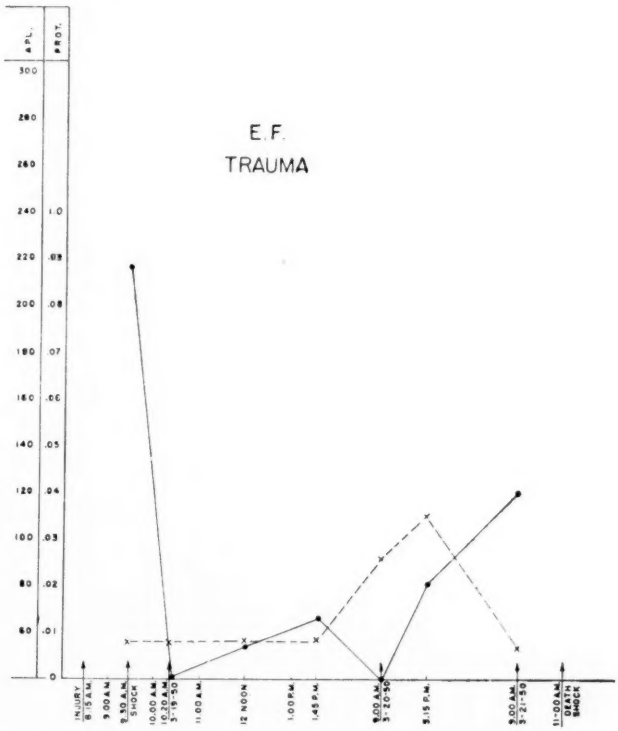

Fig. 9.—Patient struck by automobile with ultimate death due to intraperitoneal hemorrhage. The high proteolytic activity on admission to the Emergency Room rapidly fell to zero with treatment for shock. The temporary improvement of the patient corresponded with a rise in APL activity, but with the return of shock on the second day the proteolytic activity again increased while the APL activity dropped.

G. Patients on routine care (4 patients [controls]): Fig. 12 is an example of a seriously ill patient under routine regular hospital care without any definitive treatment being carried out at the time. This patient shows minimal variation in either proteolytic or APL titer.

H. Comparison of proteolytic variations with four types of treatment: Since individual cases are subject to numerous variations, a graph was prepared (Fig. 12) showing the average increase in proteolytic activity over the preliminary test in all categories where five or more cases were available for study. In the case of the postoperative state, x-ray, and ACTH treatment,

the results at ten minutes, thirty minutes, one hour, two hours, four hours, and eight hours after treatment were compared with the controls immediately preceding the treatment. In the case of the posttraumatic patients the results at thirty minutes, two hours, three hours, four hours, and over twelve hours were compared with the results on serum obtained at the time of or shortly after admission to the Emergency Room. It is apparent that with operative trauma, x-ray, and especially with ACTH treatment, there is an increase in proteolytic

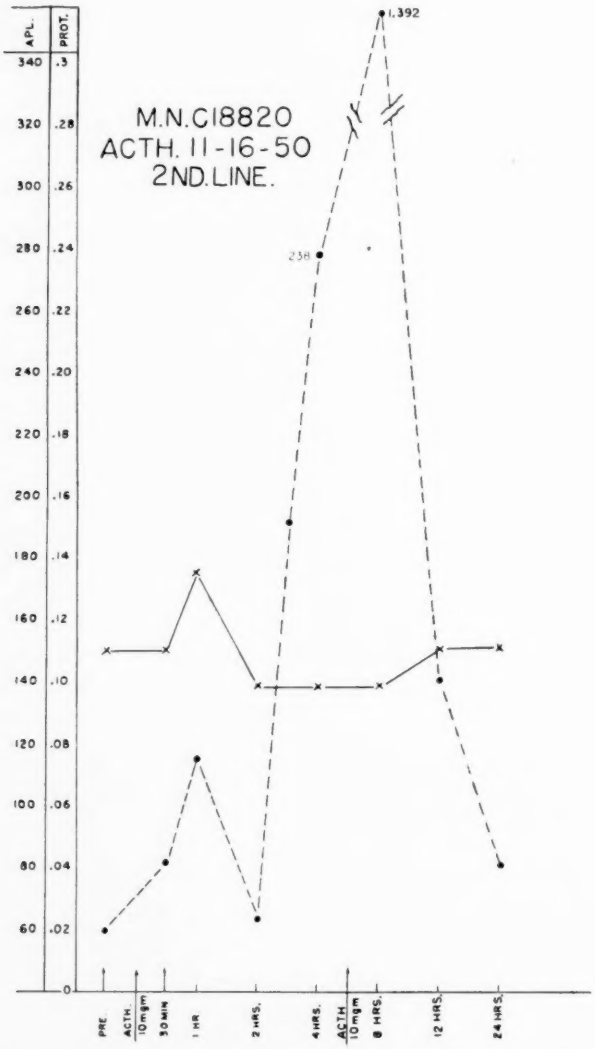

Fig. 10.—Patient treated with ACTH, first day of treatment. Note that the scale for proteolytic activity is double that used previously (0.02 mg. tyrosine rather than 0.01) which in effect decreases the apparent change in proteolytic activity. Even with this change the activity is completely off the scale as indicated by the breaks. There is a sixty-nine-fold increase in activity from the preliminary reading to the reading two hours after the second dose of 10 mg. ACTH.

activity above the preliminary proteolytic activity. In contrast, the first blood drawn after trauma showed the highest proteolytic activity, whereas subsequent specimens showed progressively less activity.

DISCUSSION

The variations in proteolytic activity with streptococcal infections are easily explained on the basis of an increase in serum antistreptokinase which is precipitated in the euglobulin fraction. Another possibility to be considered is an actual variation in the plasminogen itself. This possibility is supported by the fact that the streptococcal fibrinolysin test was not positive in two cases

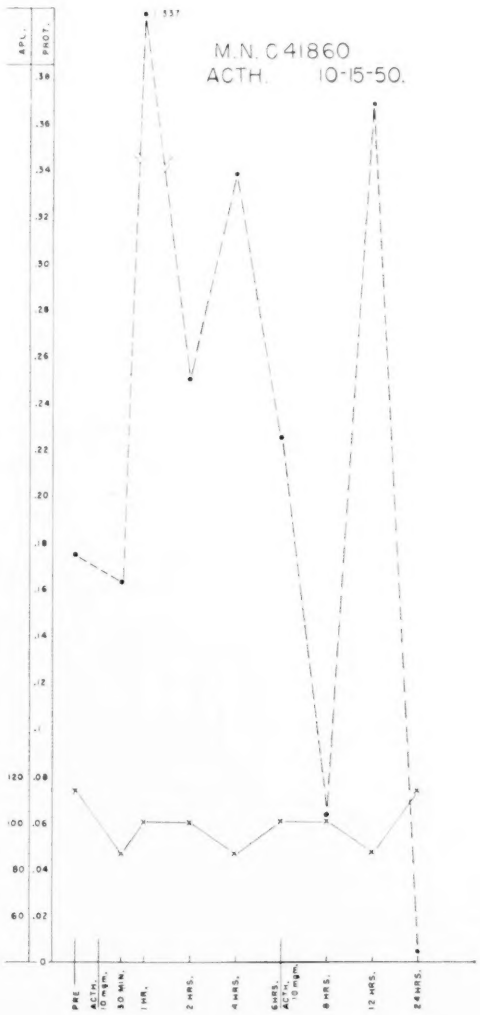

Fig. 11.—Another patient on the second day of ACTH treatment. Note that the preliminary level is already extremely high, 0.18 mg. tyrosine, much higher than any other reading obtain except with ACTH. There is once again a marked increase to 1.337 mg. tyrosine.

(No. 7 and No. 15) with significant decrease in the streptokinase activated proteolytic enzyme. This explanation would, of course, necessitate the assumption that fibrinolysin and proteolytic enzymes are not identical. Preliminary work with staphylokinase as an activator indicates that they are indeed different (data to be published).

The variations in other diseases (Table II) are much less obvious, and from the evidence of Table III are probably correlated with the variations in the APL material. These results led to the studies on variations with trauma.

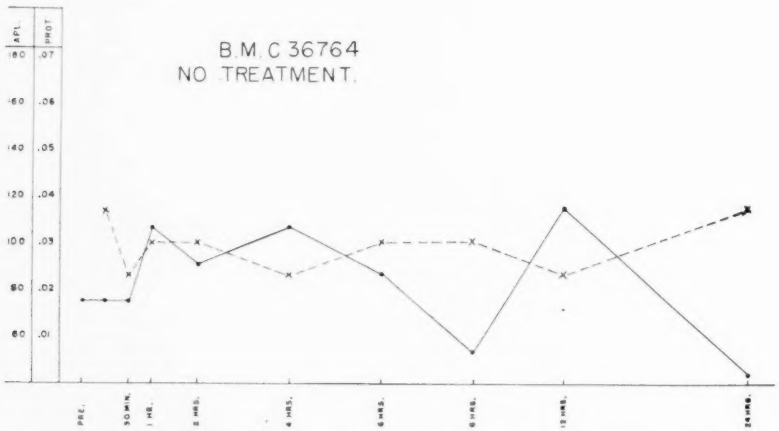

Fig. 12.—Patient with terminal carcinoma receiving only treatments for pain, infusions, and clyses. Normal individuals show even less variation within a twenty-four hour period.

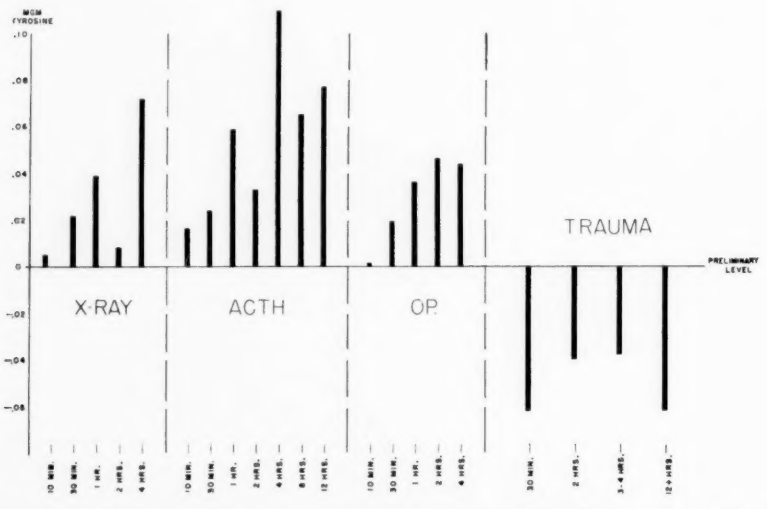

Fig. 13.—Columnar graphs to indicate variations in proteolytic activity with the three main types of treatment studied and with trauma. The base line marked preliminary level, in the case of x-ray, ACTH, and operation, indicates the proteolytic level before treatment was begun. In case of trauma this was the first specimen obtained in the Emergency Room before any treatment. Note that the ACTH and cortisone patients show the greatest rise. This includes patients who did not have an eosinophile response as well as those who did have adequate dosage. Only trauma shows a decrease in level with treatment.

It is clearly demonstrated that there is a definite increase in spontaneous proteolytic activity with trauma. This is associated in almost every instance with a decrease in APL activity. The activation of proteolytic enzymes is possibly due to tissue kinases[36] released by tissue injury. With the evidence of increase in proteolytic activity preoperatively, however, and especially in excitable patients, the possibility that this effect is due to suprarenal stimulation must be kept in mind. That suprarenal stimulation by ACTH or the adrenal hormones themselves (cortisone) will produce a marked increase in proteolytic activity is confirmed by the injection of these materials. However, it should be noted that with these substances there is not the associated inverse change in the APL activity so clearly seen with trauma; this would indicate a possible difference in the mechanism. Furthermore, adrenal hormones may still act through liberation of tissue kinases since they are known to cause rapid breakdown of tissue, especially certain white blood cells.

The inverse relationship between the levels of proteolytic enzyme and the APL can be explained on the basis of known chemical and biological facts. It has been shown that there is a 1:1 inhibition by a molecule of soy bean inhibitor acting on a molecule of trypsin.[37, 38] Injection of soy bean inhibitor intravenously has been shown to decrease the fibrinolytic activity of mouse serum and the proteolytic activity of animal serum in vitro.[39] By contrast, increase in proteolytic activity in vitro causes a decrease in APL activity of the serum. Injection of trypsin intravenously in various animals, and chymotrypsin in humans, or SKP in humans for treatment of empyema results in a temporary decrease in the APL activity of the serum and subsequently, after repeated injections (14+ days), an increase in APL activity (unpublished data[40, 41]). It is not difficult to correlate these known facts with the findings in these experiments.

It is possible that an increased proteolytic activity is of importance in the maintenance of the shock state as suggested by the incoagulability of blood in death from shock. Control of this increased proteolytic activity might aid in the control of shock. Such therapeutic control of the increased proteolytic activity might be attained by preliminary elevation of the APL titer or by the immediate injection of known inhibitors.

This data would indicate that in cases where there is eventually a rise in APL material, that it is secondary to a rise in spontaneous proteolytic activity. In the case of malignant tumors, this would presuppose the production and excretion of kinases for plasminogen into the serum, or of active proteolytic enzymes in some quantity. A less likely supposition is that the whole phenomenon is due to adrenal stimulation.

CONCLUSIONS

There is a definite decrease in proteolytic activity of serum as activated by streptokinase in patients who have had streptococcal infections.

There is an inverse relationship between proteolytic and APL activity of serum of patients with other diseases.

Physical injury of several types produces an increased proteolytic activity in serum with a simultaneous decrease in APL material. These types of trauma produce an eventual increase in APL material in most instances.

ACTH and cortisone cause a marked increase in spontaneous proteolytic activity of serum without a simultaneous decrease in the APL activity.

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EPIDEMIC INFANTILE DIARRHEA ASSOCIATED WITH ESCHERICHIA COLI 111, B_4

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DURING the past six years the medical literature of Great Britain and Europe[1, 3, 4, 5, 6, 7, 8, 9, 10] has carried a number of interesting accounts of the association of a special serologic type of coliform organism with cases of epidemic diarrhea of the newborn and of infants. The same organism is known to occur in this country in outbreaks of infant diarrhea,[1] but, to date, an account by Neter and Shumway[2] of its isolation from five sporadic cases is the only clinical data to appear in our literature.

In the present study the history of a hospital outbreak of infant diarrhea is reported in which the special coliform organism, *Escherichi coli* 111, B_4,§ was isolated from a large percentage of diarrheal infants. The clinical aspects of the cases are presented, together with the results of treatment with chloromycetin and other agents.

HISTORY OF THE OUTBREAK

During the month of August, 1950, five cases of classical "diarrhea of the newborn" occurred in the well-baby nursery of St. Mary's Hospital. After routine bacteriologic investigation failed to demonstrate the presence of any of the well-known enteric pathogens, special attention was paid to the coliform flora, and EC-111 was identified in four cases. During the subsequent six-month interval, sixty-one cases of diarrhea occurred in the Newborn and Pediatric Services, EC-111 being cultured from the stools in forty-one cases; of the nineteen other infants presenting the typical clinical picture, the diarrhea was considered to be a complication of other disease in eight (penumonia, staphylococcus food poisoning, feeding problems, etc.), while the other eleven presumably represented victims of the epidemic. In all but seven infants, the infection was apparently acquired in the hospital as indicated by the fact that diarrhea developed within one week after birth or within ten days following the newborn infant's original discharge from this hopsital. Table I compares the incidence of the disease with the infant population of the two services concerned during the period of the outbreak; the low but constant rate of clinical diarrhea indicates the stubborn nature of the disease-producing factor, despite every effort to eliminate it from the infants' environment.

All infants suffering from diarrhea were bottle fed. Formulas are prepared on the pediatric floor, whence they are dispensed to the pediatric patients and to those in the well-baby nursery on the obstetric floor immediately below. No break in aseptic technique could

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§*Escherichia coli* 111, B_4 has been called *Bacterium coli* var. Neapolitanum, *B. Coli alpha*, *B. Coli* D_{433}, *B. coli* B. G. T., but the former name is favored by the editors of the Brit. M. J.[11] in the interests of international understanding. The abbreviation EC-111 is used in this paper.

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be demonstrated in the processing of the formulas or supplemental feedings, nor was the organism ever isolated from the stools from any doctors, nurses, or attendants caring for the infants; a total of ninety-three fecal specimens were obtained over a period of four months from these contacts. Repeated cultural studies of the materials involved in the preparation of the formulas and of the milk used, both before and after sterilization, were uniformly negative. In an attempt to locate the source from which the organism was being distributed to the hospital's infant population, stools of all children under one year of age admitted to the Pediatric Service during the months of September to November, inclusive, were cultured for EC-111 (see Table II). Seven infants harbored the organism in their stools; these seven were suffering from various diseases, including pyloric stenosis (one), upper respiratory infection (two), appendicitis, (two), and pyelitis (two). In one of the appendicitis cases *E. coli* was isolated from the peritoneal exudate at operation, but unfortunately was not tested with typing serum.

TABLE I. INCIDENCE OF DISEASE CASES

	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN.	FEB.	TOTAL
Births	269	243	241	241	242	238	201	1,675
Admissions to pediatrics	127	115	148	174	142	174	102	982
Cases of diarrhea	6	18	10	7	6	7	10	64
Cases positive for EC-111	5	16	8	6	2	3	5	45
Deaths	1*	2*	1*	1	0	1	0	6
Infants examined (stools)	6	19	16	32	27	82	31	213

*Premature infants.

TABLE II. SUMMARY OF FECAL CULTURE STUDY

A. NO DIARRHEA	MORTALITY	CASES	B. DIARRHEA		MORTALITY	CASES
EC-111 not found (pediatrics)	0	142	EC-111 found		6	45
EC-111 not found (adults)	0	93	Prematures	9		
EC-111 found (pediatrics)	0	7	Newborn	9		
	0	242	Others	27		
			EC-111 not found (pediatrics)		0	11
					6	56

No systematic attempt was made to isolate EC-111 from the infants' throats, but during the latter part of the epidemic, throat cultures were made in twelve cases, five being positive for EC-111; only one of these five infants had been known to vomit during the course of his illness. The presence of the organism in the throats of affected infants has been reported by Magnusson et al.[10], and by Neter and Shumway.[2]

Control measures to suppress the outbreak included severe restrictions on the number of visitors permitted to the Obstetrical and Pediatric Services, rigid supervision of all feeding and handling techniques by the nursing and medical staffs, and prompt segregation of all questionable and frank cases of diarrhea; in addition, patients admitted to the Pediatric Service were carefully screened as possible carriers.

There were six deaths, four of them in premature infants, while the other two were 6 and 16 weeks old, respectively. Necropsy was performed on four of these, but no significant lesions were found either in the intestinal tract or elsewhere. This lack of anatomic pathology in the victims of this form of infectious diarrhea has been noted by several observers.[3, 4, 8]

DIAGNOSIS AND TREATMENT

The only constant and characteristic finding in epidemic diarrhea of the newborn is the diarrhea itself plus the consequent dehydration, electrolyte imbalance, and prostration. Diarrhea is considered to be present if more than four stools are passed per twenty-four hours, whether liquid or formed. There is

always some fever present, ranging in our series from 99.8° F. to 105.2° F.; leucocytosis is usually present with a moderate or marked elevation of granulocytes; there is no constant pronounced left shift or other helpful hematologic observation.

Once the nature of the outbreak has been suspected, search for EC-111 should be performed routinely; other pathogens such as organisms of the Salmonella and *Shigella* groups should not, of course, be overlooked. The presence of EC-111 can be detected readily, since, characteristically, it appears in almost pure growth on MacConkey's and blood agar media when cultured from diarrheal stools, and produces a somewhat seminal odor on the latter medium. The relative proportion of EC-111 to other coliform organisms decreases rapidly as an infant's condition improves.

The mortality rate in our series is relatively low as compared with that reported in similar epidemics during the past several years in which the rate ranged from 19 to 44 per cent;[1, 3, 4, 14] this is probably due to the prompt administration of antibiotics which have proved to be rather specific for EC-111 in vitro,[2, 13] as well as in vivo.[15] As will be mentioned in a later section, the organism isolated in this epidemic was found to be quite sensitive to chloromycetin and aureomycin, the former being selected as the drug of choice because of the greater ease of administration. Standard dosage was 50 mg. per kilogram of body weight per twenty-four hours, divided into six doses and given in a few cubic centimeters of formula as a special feeding; in infants unable to swallow, perforated capsules of the drug were inserted rectally. Supportive treatment was considered very important, careful attention being paid to fluid and electrolyte balance, occasionally supplemented by whole blood transfusions. Streptomycin was used in a few instances, without substantial benefit, confirming the opinion expressed by Holzel and associates.[12] The course of the disease averaged fifteen to twenty-one days in cases considered to be true "diarrhea of the newborn"; in contrast to this protracted course, symptomatic or secondary diarrhea occurring in the same age groups was usually controlled in five to seven days.

BACTERIOLOGY

During the early part of this study, fecal specimens in glycerine were mailed to the Lansing laboratories of the Michigan Department of Health, 70 miles distant. It was determined there that *Escherichia coli* 111, B_4 was present in the diarrheal specimens, while *Salmonellae* and *Shigellae* were not found. Thereafter the isolation and recovery of EC-111 from infant specimens were carried out in the laboratories of St. Mary's Hospital. The methods used were aimed primarily at exposing the presence of the special coliform bacterium and were not optimal for recovery of *Salmonellae* or *Shigellae*. However, the presence in large numbers of organisms of either of the latter groups would, undoubtedly, have been detected in a fecal specimen.

All stools were collected from the infant diapers and placed in clean paper containers. They were carried to the laboratory shortly after collection and streaked directly, without dilution in broth or saline, onto the surface of MacConkey and blood agar plates. A straight platinum wire was used for distributing the specimens on the surface of the plates. The few specimens taken from the throat and nasopharynx by cotton swabs were inoculated on MacConkey's, and then transferred to blood agar.

The peculiar odor produced by EC-111 organisms, both in a stool specimen and on artificial media, has been noted by several investigators.[1, 8] Bray[3] found that he could not

place complete reliance on the production of a seminal odor by all strains; nevertheless, the odor, when present, was helpful in detecting colonies on agar plates. The gray, nearly flat, nonhemolytic colonies of this organism have another characteristic which is helpful in recognizing them. On blood agar they are slightly sticky to touch with a wire. In time a considerable degree of skill was acquired in the recognition of EC-111 colonies, particularly from blood agar. The majority of our isolations were made on this medium. Only rarely did the MacConkey plates prove helpful. The special coliform could, of course, be isolated from the MacConkey agar, but the distinctive characteristics developed on blood agar were lacking.

It was noted that a stool specimen taken during the acute diarrheal stage frequently yielded a nearly pure culture of EC-111. On such plates the seminal odor was strong. Following treatment with chloromycetin, there was a rapid change in the flora. The EC-111 organisms disappeared and were replaced, usually, by other coliforms, streptococci, yeast, and staphylococci.

Colonies displaying the special characteristics of EC-111 were inoculated onto Kligler or agar slants and were then incubated for eighteen to twenty hours. The slants were then mailed to the central laboratories of the Michigan Department of Health where serological and biochemical studies were made.

All cultures from the St. Mary's Hospital Laboratory were examined by slide agglutination technique with two OB sera (see Kauffmann and Dupont[1] for details of serology), prepared in rabbits with living strains of *E. coli* 111, B_4 and *E. coli* 55, B_5, 6 (*Bact. coli* beta), respectively. The stock strains used for preparation of sera were furnished by Dr. C. Giles, then in Aberdeen, Scotland. Our experience has shown that a strong, instant agglutination with OB serum of EC-111 may be accepted as positive evidence of the identity of the culture. However, all cultures that were strongly agglutinated were streaked to plain agar medium. Single colonies were selected by the aid of OB serum, and transfers were made to veal infusion broth and to triple sugar agar. Growth from the latter medium was transferred to fourteen different carbohydrate substrates and to other test media. Observation of carbohydrates in which fermentation was delayed was prolonged for thirty days.

Incubation of broth cultures was made for eighteen hours, then the broth was heated for two to two and one-half hours at 100° C. in flowing steam. Formalin in 0.5 per cent concentration was added as a preservative. The heated broth culture constituted the O antigen for tests with *E. coli* O-111 serum. All cultures which gave a strong agglutination in OB serum were tested in an O serum prepared in rabbits with heated *E. coli* 111, B_4 culture. Without exception, all agglutinated to the titer of the serum.

Two strains of EC-111 isolated from diarrheal infants in St. Mary's Hospital were taken at random for serologic study. Both OB and O serums were prepared for both organisms. Reciprocal absorption studies, made with these cultures and their sera, demonstrated that the organisms were similar in both surface and O antigens. Similar absorption studies were made with each of the St. Mary's strains, and their sera, together with the Giles strain of EC-111 and its specific O and OB sera. A complete similarity of antigenic structure between the St. Mary's and Giles cultures was indicated by the absorption and agglutination tests.

Biochemical studies on the EC-111 cultures isolated during the outbreak revealed a very uniform activity on the part of the various strains. All gave typical *Escherichia coli* IMVC reactions; that is, all formed indol, were methyl red positive, Voges-Proskauer negative, and failed to grow on Simmons' citrate agar. A typical culture fermented the carbohydrates arabinose, xylose, rhamnose, maltose, lactose, mannitol, and trehalose rapidly. Fermentation of dulcitol, sorbitol, and sucrose was usually delayed. Glucose, the only carbohydrate with a Durham insert, was fermented rapidly with production of acid and gas. The carbohydrates adonitol, inositol, and salicin were not fermented in thirty days. Urea was not hydrolyzed in the medium of Stuart and Rustigian; only slight H_2S was evident on lead acetate paper strips over peptone water. All cultures were nonmotile in semisolid agar.

The reaction of strains of *Escherichia coli* 111, B_4 in salicin broth may be of epidemiological significance. Salicin fermenting strains were uniformly sensitive in vitro to streptomycin, while nonfermenters were uniformly resistant. Ferguson, Jennings, and Gottshall[15]

tested eight strains from the outbreak at St. Mary's Hospital and found them all to be nonfermenters of salicin; they were highly resistant to streptomycin and increasingly sensitive to chloromycetin, terramycin, and aureomycin, in the order named.

The examination of fecal specimens from nurses, doctors, and attendants in contact with infants in St. Mary's Hospital was made by the Division of Laboratories of the Michigan Department of Health. Specimens were shipped in 30 per cent glycerine containers and were in transit about twenty-four hours. They were cultured on blood and MacConkey agar, as well as SS and bismuth sulfite plate media. Attempts to detect EC-111 were made by slide agglutination technique with specific OB serum. No isolation of the special coliform organism was made from any specimen.

Only two serum specimens from infants were examined for agglutinins for *E. coli* 111, B_4. The first was taken from infant D. H. fourteen days after the onset of diarrhea. A heated suspension (O antigen) of EC-111 was agglutinated in a dilution of 1:20 of the serum; a living suspension was not agglutinated to any extent by the serum.

The second specimen was obtained from Baby O. twenty-six days after onset of symptoms. A heated suspension of EC-111 was agglutinated in a dilution of 1:256, while living organisms were agglutinated to a titer of 1:16.

It has been noted by one of us, from examinations of serum taken in other outbreaks, that serum agglutinins for living suspensions of EC-111 are not high. Agglutinins, if present, are detected with heated suspensions. This is in agreement with the experience of Giles, Sangster, and Smith.[6]

DISCUSSION

It seems clear from the literature on epidemic diarrhea of the newborn that a variety of agents may be responsible for this syndrome. This view has been admirably summarized by Kirby, Hall, and Coackley,[14] who feel that there are perhaps six main groups of the syndrome, with one of which EC-111 is associated. According to these authors, the symptoms of the latter group are pronounced loss of weight, anorexia, toxemia, diarrhea and vomiting, severe dehydration, and a tendency to relapse. Adults are not affected, but the mortality rate is high in infants.

In the series of cases under consideration in this paper the symptoms noted by Kirby and co-workers[14] were present but not as constant characteristics of individual cases. Weight loss, as one would expect, was usual, anorexia was frequent, toxemia was variable, vomiting was not present in all cases; the constant symptoms were severe diarrhea and dehydration. Adults were not affected.

The possibility of virus etiology[16] was not ruled out in the present study, as well as the possibility that EC-111 represents a symbiotic factor related to the responsible virus. Bray[3, 4] also was careful to point out that he had not excluded the possibility of a viral agent. In fact, with the exception of a report in the paper of Taylor, Powell, and Wright,[5] in which careful attempts were made to demonstrate recovery of a virus from four fatal cases, virus etiology has not been ruled out by any of the authors who have reported the isolation of this bacterium. However, certain facts tend to confirm our impression of a causal relationship between EC-111 and the cases of diarrhea. These are as follows: (a) The organism has been found in nearly pure culture, in the absence of known pathogens, during the acute stage of diarrhea, while later, during the convalescent period, it has been replaced by other enteric bacteria.[8] (b) Agglutinins for *E. coli* 111, B_4 have been demonstrated in the serums of infants recovering from

a bout of epidemic diarrhea.[6, 8] (c) *E. coli* 111, B_4 has been recovered from the upper respiratory tract of diarrheal infants who were excreting the organism in feces.[2, 10] (d) Diarrheal infants, when treated with aureomycin, have recovered clinically at the same time *E. coli* 111, B_4 disappeared from the nasopharynx and feces.[10] (e) The special coliform has been demonstrated in the stools of only a small percentage of normal adults and normal infants.[3, 5, 6] (f) One instance is on record in which a nondiarrheal infant was fed *E. coli* 111, B_4. Diarrhea and weight loss were produced within twenty-four hours. The organism was demonstrated in small numbers in the throat and in large numbers in the feces, but not in the nasopharynx. Within forty-eight hours *E. coli* 111, B_4 was found in very large numbers in the throat, nasopharynx, and feces. Treatment with terramycin resulted in clinical improvement and the disappearance of the organism within forty-eight hours.[2]

Isolation of the organism from the throats of several infants indicates the possibility of spread through a crowded nursery by droplet infection, although it is undoubtedly transmitted most often via artificial feeding equipment or by nursing personnel. As in all such hospital outbreaks, this epidemic was characterized by extreme persistence, which is not remarkable in view of the resistant character of coliform organisms to drying, sunlight, and other agents. It should be recalled that more severe epidemics have necessitated complete closing of hospital maternity departments for considerable periods of time.

SUMMARY

During a seven-month period, fifty-six cases of "diarrhea of the newborn" occurred in a hospital Pediatric and Maternity Service, among a population totaling 2,698 children; in 80 per cent of affected infants a specific coliform organism was isolated. The mortality rate was 10 per cent.

The special cultural properties of this organism are described, including sensitivity to available antibiotics.

Examination of the stools of 146 infants not suffering from the disease demonstrated the organism in 4.7 per cent of the cases.

Similar investigation of ninety-three adult contacts during the same period failed to demonstrate the organism in any of them.

A relatively low mortality rate, as compared with outbreaks of similar character reported heretofore, is believed to be due to the use of chloromycetin plus careful attention to supportive therapy.

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QUANTITATIVE ANTIBODY STUDIES IN MAN

II. The Relation of the Level of Serum Proteins to Antibody Production

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THE relation of the level of serum proteins to the ability to produce circulating antibodies has been investigated by several groups of workers. In animals depleted of protein by dietary restriction or plasmapheresis, antibody responses have been observed to be impaired[1-6] or normal.[7] In human beings with hypoalbuminemia, with or without hyperglobulinemia, antibody responses have been observed to be decreased[8, 9] or normal.[10] In studying human beings following streptococcal infections, it has been noted that the group of patients with the highest gamma globulin levels also had the highest antibody titers.[11] The purpose of this communication is to report studies on antibody responses in twelve patients with abnormal levels of serum albumin and globulins, using quantitative immunochemical techniques.

MATERIAL AND METHODS

The method of Heidelberger and MacPherson[12] was used to determine the amount of precipitable antibody nitrogen. Preliminary decomplementation and removal of C-antibody was carried out on all sera before analyzing the specific precipitates.[13, 14] Sera were decomplemented with crystalline ovalbumin and rabbit anti-ovalbumin serum. C-antibody was removed with C-polysaccharide derived from and type III or a type VII pneumococcus.†

Patients used in this study were from the hospital and clinic population of the Presbyterian Hospital. The patients were on regular diets, and no therapy known to influence antibody levels was given before or during the test period. A preliminary bleeding was obtained, followed by the subcutaneous administration of 0.08 mg. each of type-specific pneumococcus capsular polysaccharides I and II. Postimmunization bleedings were obtained at three weeks. Results of antibody determinations are reported as micrograms of precipitable antibody nitrogen per 4 ml. of serum.

Total serum albumin and globulin determinations were done by a modification of the Howe method.[15, 16] Euglobulin determinations were not done unless the total serum globulin was at least 3 grams per cent. The normal range of values with this method are: albumin, 5.7 to 4.7 grams per cent; globulins, 2.5 to 1.3 grams per cent; euglobulins, 0.4 to 0.1 grams per cent.[21]

The concentration of pneumococcus anti-polysaccharide antibody attained in man is too low to be specifically detected on electrophoretic examination. Therefore, no change in electrophoretic pattern would result from immunization. Electrophoretic analyses were carried out on seven of the immunized patients.‡ Sera were diluted 1:3 with a buffer containing

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‡Electrophoretic analyses were completed in the laboratory of Dr. Dan H. Moore.

0.15 M NaCl and 0.02 M phosphate, ionic strength 0.2, at pH 7.4. A seventeen to twenty-eight hour dialysis of the serum at 4° C. against 500 ml. of the buffered saline was carried out before the electrophoretic analyses were done.

RESULTS

Previous quantitative immunochemical studies have indicated that normal individuals vary considerably in their response to the administration of pneu-

TABLE I. RELATION OF THE LEVEL OF SERUM PROTEINS TO ANTIBODY PRODUCTION

PATIENT	GRAMS PER CENT			ANTIBODY TO**	MICROGRAMS OF ANTIBODY NITROGEN PER 4 MILLILITERS OF SERUM	
	ALBUMIN	GLOBULIN	EUGLOBULIN		PRE-IMMUNIZATION	3 WEEKS POST-IMMUNIZATION
76 Hodgkin's disease	2.9	2.4	*	C VII	64	138
				S I	1	0
				S II	1	0
36 Bronchiectasis	4.5	3.4	0.5	C III	105	74
				S I	0	36
				S II	0	56
63 Lupus erythematosis	3.8	3.8	1.1	C VIII	27	16
				S I	1	6
				S II	0	8
60 Cirrhosis of the liver	4.8	3.1	0.7	C VII	32	33
				S I	0	2
				S II	3	13
55 Cirrhosis of the liver	2.3	4.3	1.5	C VII	59	70
				S I	0	68
				S II	0	93
54 Cirrhosis of the liver	3.8	3.6	0.4	C VII	63	82
				S I	0	29
				S II	0	61
53 Cirrhosis of the liver	3.4	2.8	0.0	C VII	24	19
				S I	2	7
				S II	2	30
95 Multiple myeloma	4.1	1.2	*	C VII	12	4
				S I	0	0
				S II	0	16
75 Multiple myeloma	3.1	8.7	0.3	C VII	3	3
				S I	0	0
				S II	0	0
68 Multiple myeloma	1.7	8.9	*	C VII	1	3
				S I	0	2
				S II	0	1
42 Multiple myeloma	3.4	4.1	1.5	C III	12	2
				S I	0	6
				S II	0	2
13 Diabetes mellitus	3.5	3.1	0.5	C III	27	27
				S I	0	69
				S II	1	36

*Euglobulin determination not done.
**Letter and number refer to the polysaccharide precipitinogen used.

mococcal capsular polysaccharides.[14, 17, 18] Postimmunization titers usually range between 10 and 60 μg of antibody nitrogen per 4 ml. of serum. Some individuals, however, form little or no detectable antibody, and a few subjects have formed in excess of 100 μg per 4 ml. liters of serum. The levels of C-antibody present in most human beings are between 10 and 60 μg of antibody nitrogen per 4 ml. of serum, and the values for any one individual remain relatively constant over long periods of time.

In Table I, data are presented on twelve patients having diseases which are often associated with abnormal levels of serum proteins. The pre- and post-immunization antibody titers are given. Values for total serum albumin, total serum globulins, and serum euglobulins, obtained on sera procured during the period of active immunization are also included. It can be seen that there is no apparent relation between the degree of depression of the albumin, the degree of elevation of the globulins, or the degree of elevation of the euglobulins, and the amount of antibody globulin produced. Among the four patients with cirrhosis of the liver, three had hypoalbuminemia, three had hyperglobulinemia, and two had elevations in euglobulins. All four patients exhibited a normal antibody response. Among the four patients with multiple myeloma,* all had hypoalbuminemia, three had hyperglobulinemia, and at least one had an elevation in euglobulins. Each patient had low levels of C-antibody and each patient demonstrated a poor antibody response to the capsular polysaccharides.

Antibody to the pneumococcus in the horse, rabbit, and monkey has been shown to exhibit the electrophoretic mobility of gamma globulin.[19a, b] An attempt was made, on the basis of electrophoretic patterns, to correlate the serum level of gamma globulins with the ability to produce antibody globulin. In Table II the electrophoretic analyses on serum samples from seven of the patients included in Table I are presented. Comparing the data in the two tables, it becomes apparent that there is no correlation between the ability to maintain an elevated serum gamma globulin level and the ability to produce antibody globulin. Patient 75 is an example of an individual with an exceedingly high gamma globulin level, i.e., 66 per cent of his total serum proteins, but with no detectable production of antibody globulin.

COMMENT

A number of papers have appeared indicating that animals[1-6] and man[8, 9] with hypoalbuminemia, with or without hyperglobulinemia, have impaired ability to produce circulating antibody. Other investigators have been unable to confirm this observation in animals[7] or in man.[10] Alteration in serum proteins in animals induced by prolonged dietary restriction or plasmapheresis is not strictly comparable to the disturbances in protein metabolism present in the patients used in this study. Baleh has studied the relation of nutritional deficiency to antibody production in man,[10] using a method which lends itself to quantitative interpretation. He found no relation between diphtheria antitoxin production and the initial level of antitoxin, the total serum protein, the total

*Patient 68 was made available through the courtesy of Dr. Chester Southam.

TABLE II. ELECTROPHORETIC DISTRIBUTION OF PROTEINS IN SERA FROM SEVEN OF THE IMMUNIZED PATIENTS INCLUDED IN TABLE I*

PATIENT	DILUTION	PER CENT CONCENTRATION				A/G RATIO
		ALBUMIN	GLOBULINS			
			ALPHA	BETA	GAMMA	
76 Hodgkin's disease	1:3	43.2	18.9	8.4	29.5	0.76
55 Cirrhosis of the liver	1:3	31.2	9.7	10.8	48.3	0.45
54 Cirrhosis of the liver	1:3	41.5	17.9	8.5	32.1	0.71
53 Cirrhosis of the liver	1:3	58.5	11.3	10.4	19.8	1.41
95 Multiple myeloma	1:3	67.3	10.9	10.9	10.9	2.06
75 Multiple myeloma	1:3	26.1	3.3	4.6	66.0	0.35
42 Multiple myeloma	1:5	41.8	12.5	12.5	33.2	0.72

*Dialysis time: seventeen to twenty-eight hours; 3 ml. diluted serum dialyzed against 500 ml. of buffer.
Current: 20 milliamperes.
Time of runs: average of two and one-half hours.
Buffer: pH 7.4; 0.15M in NaCl, 0.02M in phosphate; ionic strength 0.2.
Magnification used for area determinations, 3:1.
All measurements made on descending pattern.

serum albumin and the total serum globulins. Although the patients in the present study were not protein depleted, the same lack of relationships holds between values for serum protein components and the precipitin production.

From studies on human sera[20, 21] it has become apparent that there is no sharp correlation between the disease present and the electrophoretic pattern. There is an extraordinary variability in the distribution of the fraction of the globulins, and the value obtained for total serum globulins provides no information on the amount of each fraction present. By combining electrophoretic and salt fractionation methods of examination of serum proteins with quantitative antibody determinations, it has been possible to show that, in man, the ability to maintain elevated total serum gamma globulins bears no relation to the ability to produce antibody globulin to pneumococcus polysaccharides.

In view of the recent work in animals on the role of the plasma cell in antibody production,[22-26] it is of interest that the four patients in this study with multiple myeloma had distinctly impaired antibody responses. The only other quantitative data in the literature on the antibody response in multiple myeloma indicate a normal diphtheria antitoxin production in the one patient examined.[10] Additional information will be required to determine whether the paucity of precipitin production in multiple myeloma is confined to pneumococcal polysaccharide antibody.

SUMMARY

1. Antibody production was studied in twelve patients with abnormal levels of serum proteins using quantitative immunochemical techniques.

2. There did not appear to be any relation between total serum protein, total serum albumin, total serum globulins, total serum euglobulins, the electrophoretic pattern and the ability to form antibodies.

3. In the patients studied, the ability to maintain elevated total serum gamma globulins bore no apparent relation to the ability to produce antibody globulin.

4. Four patients with cirrhosis of the liver had normal antibody responses.

5. Four patients with multiple myeloma had notably poor antibody responses and unusually low titers of C antibody.

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THE EFFECT OF TRYPSIN ON HEMAGGLUTINOGENS DETERMINING EIGHT BLOOD GROUP SYSTEMS

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THE saline agglutination, albumin-plasma, indirect antiglobulin, and enzyme-treated cell methods are all now used to determine Rh antibody content of serums. An additional method has been proposed by one of us (L.J.U.), namely, the trypsinated cell indirect antiglobulin method. It has been shown[1] that this method, when used to titrate serum containing Rh_0 univalent antibodies, in general, yields slightly higher titration figures than other methods tried by us, so that univalent Rh_0 antibodies could be detected even when present in such low concentration that they were below the threshold of the other methods. The purpose of this study is to determine the effect of trypsin on other hemagglutinogens besides Rh.

MATERIALS AND METHODS

The methods of titration mentioned above were used to titrate antibodies specific for factors belonging to eight blood group systems, namely Rh-Hr, ABO, MNS, P, Lewis, Lutheran, Kell-Cellano and Duffy. Wherever possible, two or more examples of antiserums of each specificity were used† in the titration experiments. Among the rarer serums tested there were four examples of anti-Kell, three examples each of anti-Lewis and anti-P, two examples each of anti-M, N, S and Duffy, one example each of anti-Lutheran and anti-Cellano.

The red blood cells used for each set of titrations were obtained from the same individual and were known to be of desired specificity. In addition, cells lacking the factor under investigation were used as controls. All cells were washed four times in normal saline, packed, and then divided in two parts; one part was reconstituted as a 2 per cent saline suspension and used as untreated cells for the albumin-plasma, saline agglutination, and indirect antiglobulin titrations; the other part was treated with trypsin and used for comparative titrations in saline and by the indirect antiglobulin method. The trypsinated cells were prepared as follows: to nine parts of four times washed‡ packed cells, one part of 1 per cent trypsin (Difco) in standard phosphate buffer solution was added, mixed, placed in a water bath at 37° C. for one hour, and then, after washing once with normal saline to remove the trypsin, diluted in saline to make a 2 per cent suspension.

All titrations were carried out in the usual fashion. The saline agglutination and trypsinated cell methods were carried out as follows: one drop of progressively doubled saline dilutions of serums was put into each of a series of small test tubes (8 mm. in diameter). The same pipette was used throughout for accuracy, and by thoroughly rinsing it with fresh saline solution between each use, "carrying over" of serum was avoided. One drop of 2 per cent red cell suspension of proper type was added to each tube (untreated or trypsinated depending on the method). Incubation was carried out in a water bath at 37° C. for one

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†Serums were very kindly given to us by Drs. Abelson, Allen, Chown, Levine, Miller, Mourant, van Loghem, Vogel, and Wiener. We are greatly indebted to them for their cooperation.

‡Washing is necessary to remove the trypsin inhibitor present in plasma.

hour. The sediment in each tube was then examined for clumping under the microscope using the lowest (scanning) power. The titer was taken equal to the reciprocal of the highest dilution showing a one plus clumping of cells.

The technique for the albumin-plasma method was the same as the saline agglutination method, except that after the first hour of incubation the supernatant fluid was removed as thoroughly as possible from each tube and replaced by a large drop of a mixture of four parts of pooled oxalated plasma from Rh-positive individuals and one part of 30 per cent bovine albumin solution. After an additional hour of incubation at 37° C., the mixtures were examined for clumping as already described.

The antiglobulin test was carried out on the cell sediment in each tube of the saline agglutination titration and trypsinated cell titration where no clumping was observed at the end of the period of incubation. The technique used was as follows: The cells were washed four times, each time with at least ten volumes of normal saline solution, and then the saline removed. Two drops of antihuman globulin serum (rabbit) were added and mixed with the cells. The mixture was centrifuged lightly (500 to 1,000 r.p.m.) for one minute and then the sediment gently resuspended and examined *in the tube* for clumping and read as in the other methods under the lowest power of the microscope (scanning lens).

RESULTS

In order to appraise the effect of trypsin on hemagglutinogens, one should compare results of titrations employing the same procedures and materials throughout, but using untreated cells in one instance and trypsinated cells in the other. The saline agglutination and indirect anti-globulin methods serve this purpose, since each can be used with untreated as well as treated cells. The results of these four methods, as well as those of the albumin-plasma method when various specific antiserums were titrated are given in Tables I, II, and III.

The Rh_0, $\mathfrak{R}h_0$, rh′, rh″, rh^w, hr′, A, B, S, P, Lewis, Cellano, and Lutheran Factors.—These hemagglutinogens are made slightly more sensitive or, as in a few instances, are unaffected by trypsinization. In general, slightly higher titers resulted with specific bivalent serums (compare column 2 and 3 in Table I)

TABLE I. TITRATIONS WITH SPECIFIC BIVALENT ANTISERUMS USING UNTREATED AND TRYPSINATED CELLS CONTAINING RH-HR, A-B, S, P, LEWIS, AND LUTHERAN ANTIGENS

	METHODS AND TITERS (IN UNITS)				
	ALBUMIN-PLASMA	SALINE AGGLUTINATION		INDIRECT ANTIGLOBULIN	
ANTISERUM	(1) UNTREATED	(2) UNTREATED	(3) TRYPSINATED	(4) UNTREATED	(5) TRYPSINATED
Anti-Rh_0	128	80	256	160	320
Anti-rh′	40	24	128	64	256
Anti-rh″	1½	1	4	2	6
Anti-rh^w	16	16	32	32	32
Anti-hr′	32	30	64	128	256
Anti-A	32	32	32	64	64
Anti-A	50	32	48	50	64
Anti-B	4	4	12	4	12
Anti-B	6	6	12	6	12
Anti-S	2	4	16	8	16
Anti-S	5	2	12	6	16
Anti-P	2	2	8	4	8
Anti-P	1	0	1½	2	1½
Anti-P	8	8	16	16	16
Anti-Le^a	1½	2	2	2	4
Anti-Le^a	2	1	4	2	4
Anti-Le^a	64	20	64	64	128
Anti-Lu^a	1	2	2	2	2

TABLE II. TITRATIONS WITH SPECIFIC UNIVALENT ANTISERUMS* USING UNTREATED AND TRYPSINATED CELLS CONTAINING Rh_0, $\mathfrak{R}h_0$, rh″, hr′, hr″, A, B, AND CELLANO ANTIGENS

	METHODS AND TITERS (IN UNITS)				
	ALBUMIN-PLASMA MEDIA	SALINE AGGLUTINATION		INDIRECT ANTIGLOBULIN	
ANTISERUM	(1) UNTREATED	(2) UNTREATED	(3) TRYPSINATED	(4) UNTREATED	(5) TRYPSINATED
Anti-Rh_0†	32	1	40	160	450
Anti-Rh_0‡	16	0	20	80	96
Anti-rh″	20	4	40	64	80
Anti-hr′	32	1	80	96	150
Anti-hr″	4	2	8	8	32
Anti-A§	6	0	4	64	512
Anti-A§	24	12	64	128	512
Anti-B§	40	15	40	50	100
Anti-B§	35	0	70	80	250
Anti-Cellano	32	6	16	64	128

*Some contain bivalent as well as univalent antibodies.

†The cells used in these titrations were clumped both by bivalent and strong univalent anti-Rh_0 serum. They therefore are standard Rh_0 cells.

‡The cells used in these titrations were not clumped by bivalent anti-Rh_0 serum, but were clumped by strong univalent anti-Rh_0 serum. They therefore possessed a variant of Rh_0 ($\mathfrak{R}h_0$).

§The anti-A and anti-B serums used for these tests were obtained from stimulated donors. To one part of this serum, four parts of A and B substance (Sharp and Dohme) were added to neutralize natural bivalent antibodies.

TABLE III. TITRATIONS WITH SPECIFIC ANTISERUMS USING UNTREATED AND TRYPSINATED CELLS CONTAINING M, N, KELL, AND DUFFY ANTIGENS

	METHODS AND TITERS (IN UNITS)				
	ALBUMIN-PLASMA	SALINE AGGLUTINATION		INDIRECT ANTIGLOBULIN	
ANTISERUM	(1) UNTREATED	(2) UNTREATED	(3) TRYPSINATED	(4) UNTREATED	(5) TRYPSINATED
Anti-M	20	10	6	16	5
Anti-M	64	16	10	128	16
Anti-N	16	10	8	16	10
Anti-N	6	4	3	10	4
Anti-Kell	20	0	0	80	80
Anti-Kell	64	1	2	190	190
Anti-Kell	3	0	0	256	320
Anti-Kell	8	0	2	32	32
Anti-Fy^a	0	0	0	16	0
Anti-Fy^a	0	0	0	32	0

and definitely higher titers with specific univalent serums (Table II). It is well recognized that a variation in titer of one dilution may be due to limitations of the method. Yet it is difficult to ignore such variations when they occur consistently.

Beyond the end point of a titration, which is the highest dilutions of the serum at which cell clumping is observed, there are usually some cells which, although coated, do not form clumps, and coating of these unclumped cells can be detected by the antiglobulin test whether or not the test cells were previously trypsinated. Trypsinization causes the antigens listed above to become somewhat more readily coated not only by bivalent antibodies (compare column 4 and 5 in Table I), but also to a greater degree by univalent antibodies (Table II). Furthermore, the trypsinated cell antiglobulin method carried out in *test tubes*

and using a high titered antiglobulin serum (rabbit) yielded titers equal to or higher than other methods, especially when the antibody was of the univalent variety.

The M-N, Kell and Duffy Factors.—This group of antigens is affected in a fashion different from the first group.

Titrations using type M and type N cells were carried out by the test tube method, which is more sensitive than the well-slide technique ordinarily used for these serums and residual nonspecific agglutinins present in the rabbit serum, reacted with all cells especially when they were trypsinated. Wiener and Katz reported similar results.[2] However, the M and N factors are adversely affected by trypsin, as evidenced by a decrease in titer (Table III, columns 4 and 5). Morton and Pickles[3*] report that anti-M, N, and S serums showed a slight increase in titer and avidity of reaction when tested against specific cells treated with crystalline trypsin (Plaut Research Co.), but with all other brands of trypsin, and also with the proteolytic enzyme from *Streptomyces albus*, there appeared to be destruction of the antigen locus. Rosenfeld and Vogel[4*] report that the M and N factors are largely destroyed by enzyme treatment of red cells, because when trypsin-treated red cells are used to absorb specific antiserums, the M and N antibodies are not removed.

The effect of trypsin on the Kell factor is interesting and of a different nature. Although all four examples of anti-Kell used were univalent serums, their antibodies produced no clumping of saline suspended trypsinated Kell-positive cells in two instances and gave only a titer of two units with the other two examples. Considerably higher titers were obtained with untreated cells suspended in albumin-plasma media (Table III, columns 1 and 3). After complete removal of saline from each tube of the trypsinated cell titrations, and replacement with one drop of albumin-plasma mixture followed by incubation in the water bath at 37° C., clumping resulted and the titers were approximately the same as those obtained by the albumin-plasma method using untreated cells, showing that trypsin had not interfered with the coating of the cells.

When the antiglobulin methods of titration (Table III, columns 4 and 5) were carried out, still higher titers were obtained, but these were identical for both treated and untreated cells. This is interpreted as meaning that trypsin treatment neither affects the sensitivity of the Kell antigen nor its ability to become coated with specific antibodies. Yet, in spite of the fact that trypsinated cells are coated with univalent antibodies, they fail to clump when saline suspended. In these two respects the Kell factor is affected differently by trypsin than the Rh_0 factor. The Kell factor is not made more sensitive when used to titrate specific antiserums, whereas the Rh_0 factor is; univalent anti-Kell coats but fails to clump trypsinated saline suspended Kell-positive cells; whereas univalent anti-Rh_0 both coats and clumps trypsinated saline suspended Rh_0 positive cells.

The Duffy factor is markedly affected by trypsin; in fact, it appears to be completely destroyed and unable to react with its specific antibody.[5] Trypsinated Duffy-positive cells are unable to be coated by their specific antibody (Table III,

*The present work had been completed prior to publications of references 3 and 4.

column 5) and this explains why they are unable to form clumps (Table III, column 3). After trypsinization the Duffy factor has no ability to absorb antibodies from its specific serum. Only untreated Duffy positive cells can be coated with specific antibody, and even then such saline suspended coated cells form no visible clumps (Table III, columns 2 and 4).

The results obtained with the trypsinated cell indirect antiglobulin method when testing the Kell and Duffy antigens differ and demonstrate an additional value of this method. The method shows that Kell-positive cells are able to react with specific antibodies and absorb them from serum, whereas this is not the case with trypsinated Duffy-positive cells which are unable to react with the corresponding antibody.

Although trypsinization of erythrocytes affects different antigens differently (Table IV), it makes certain antigens more sensitive and permits detection of antibodies specific for them when present in low titer. For the detection of certain univalent antibodies, saline-suspended trypsinated cells can be used. The method yields sharp and more easily read end points, and eliminates the prozone effect found with some serums. This method can, by detection of sensitization, be

TABLE IV. A SUMMARY OF THE EFFECT OF TRYPSIN ON VARIOUS HEMAGGLUTINOGENS

SPECIFICITY OF ANTISERUM	CLUMPING OF TRYPSINATED CELLS†	COATING OF TRYPSINATED CELLS‡
Rh_o,* rh′, rh″, rh^w, hr′, hr″, A, B, S, P, Le^a, Cellano, Lu^a	slightly increased	slightly increased
M, N	decreased	decreased
Kell	none present	unchanged
Fy^a	none present	none present

*Tested against Rh-positive cells containing Rh_o (variant) as well as cells typically Rh_o-positive.

†Clumping of cells as evidenced by titration results of the trypsinated cell method compared with those of the saline agglutination method.

‡Coating of cells as evidenced by titration results of the trypsinated cell indirect antiglobulin method compared with those of the indirect antiglobulin method.

of help in the prevention of post-transfusion hemolytic reactions. A disadvantage of the trypsin method for this purpose is that it is time consuming. If cells of the prospective donor and patient are each trypsinated prior to cross-match preliminary to transfusion, a delay of about one hour results. In addition, this method cannot be used alone because failure to detect the Duffy antibody will definitely result, and if the antibodies are present in sufficiently low titer, possibly Kell, M and N might not be detected. If the trypsin method is to be used at all in the cross-match test, it must be in conjunction with other methods. These limitations also apply to using trypsinated test cells to detect sensitization of a patient.

At our Blood Bank, the cross-matching procedure consists of several tests. A mixture of two drops of patient's serum and two drops of a 2 per cent normal saline suspension of donor's cells is prepared and another similar mixture of donor's serum and patient's cells. To detect the possible presence of univalent antibodies which act only in a protein media, a second set of two similar cell-serum mixtures are prepared, with the exception that the 2 per cent suspension

of cells is made in the individual's own serum, instead of saline. These four mixtures are incubated in a water bath at 37° C. for thirty to sixty minutes, and then examined under the microscope for clumping. On the cell sediments in each of the two tubes containing the saline suspended cells, if no clumping is observed, an antiglobulin test is performed. All these tests must be negative for cell clumping if the donor's blood is to be used for transfusion. Parallel with those tests, the serum of both patient and donor is tested for the presence of atypical antibodies. This is done by making a mixture of equal parts of the serum of each of these two individuals with a 2 per cent normal saline suspension of untreated cells and a second set using a 2 per cent normal saline suspension of the same cells but trypsinated. They are incubated in a water bath at 37° C. for thirty to sixty minutes, and then examined under the microscope for clumping. The antigens present in the cells used for these sensitivity tests are known and aside from the fact that they are group O cells, as many antigens as possible are represented. It is felt that these procedures give the patient, with the least delay, the highest practicable degree of protection against post-transfusion hemolytic reactions.

SUMMARY

In order to appraise the effect of trypsin (Difco) on hemagglutinogens of known specificities, titrations of specific antiserums were carried out by five methods. Comparison of the results of these titrations shows that this enzyme does not affect all antigens in the same fashion.

The Rh_0, $\mathfrak{R}h_0$, rh′, rh″, rh^w, hr′, hr″, A, B, P, S, Le^a, Cellano, and Lu^a factors are made slightly more sensitive or unaffected by trypsinization. Except for the latter, they therefore become clumped more readily by weaker dilutions of specific serums, giving higher titration results than the same cells when untreated.

Saline suspended trypsinated cells, positive for the Rh_0, $\mathfrak{R}h_0$, rh″, hr′, hr″, A and B factors are clumped by univalent antibodies, whereas such antibodies are unable to clump the same cells when untreated unless they are suspended in protein media.

In contrast to these results, the M, N, Kell, and Duffy antigens are affected differently by trypsin treatment. Cells of types M and N are rendered less able to be clumped or coated by specific antibodies, possibly because the corresponding antigens are damaged.

The Kell antigen does not appear to be affected by trypsinization. Combination with specific antibody takes place to the same degree, whether the cells are trypsin treated or not. This was demonstrated by a comparison of results of the trypsinated cell indirect antiglobulin method with those of the indirect antiglobulin method using untreated cells. Trypsinated Kell-positive cells, although coated with specific univalent antibodies, fail to clump when saline suspended. These two findings are in direct contrast with those obtained with trypsinated Rh_0 positive cells.

The ability of the Duffy positive cells to be clumped by its specific antibody is completely lost after treatment with trypsin. This antigen seems to be

destroyed, as shown by its inability to react with and become coated by anti-Fy^a and by its inability to absorb specific antibodies from serum.

A method for cross-matching preliminary to blood transfusion which gives the patient the highest practicable degree of protection against post-transfusion hemolytic reaction is described.

ADDENDUM

Since the completion of these experiments, anti-s and anti-Kidd serums were obtained. The effect of trypsin on the antigens specific for these serums was therefore studied. It was found that the effect of trypsin on the s factor was similar to its effect on the Rh_0 factor; namely the s antigen was made slightly more sensitive (Table V). The Kidd factor however, was affected by trypsin in the same fashion as the Kell factor. Although the antibody-antigen reaction took place as demonstrated by the trypsinated cell antiglobulin test, these trypsinated coated saline suspended cells did not form clumps (Table V).

TABLE V. TITRATIONS WITH SPECIFIC ANTISERUMS USING UNTREATED AND TRYPSINATED CELLS CONTAINING S AND KIDD ANTIGENS

	METHODS AND TITERS (IN UNITS)				
	ALBUMIN-PLASMA	SALINE AGGLUTINATION		INDIRECT ANTIGLOBULIN	
ANTISERUM	(1) UNTREATED	(2) UNTREATED	(3) TRYPSINATED	(4) UNTREATED	(5) TRYPSINATED
Anti-s	4	0	8	20	40
Anti-Kidd	6	0	0	64	64

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THE EFFECT OF HEATING ON THE THROMBOPLASTIC ACTIVITY OF RABBIT BRAIN EXTRACT. A NEW TEST FOR THE DIAGNOSIS OF HEMOPHILIA*

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THE hypothesis of Alexander Schmidt that prothrombin is activated to thrombin by zymoplastic substances is now generally accepted, but that term, or its modern equivalent, "thromboplastin," remains ill defined, and there is still no general agreement concerning its source. In a study reported in 1947 one of us[1] showed that normal platelets contain not more than a trace of active thromboplastin, that little prothrombin is consumed in the clotting of either hemophilic blood or of deplateletized normal plasma, and that the addition of deplateletized normal plasma to hemophilic blood increases the utilization of prothrombin. From these observations, it was concluded that platelets do not contain thromboplastin, but on lysis liberate an agent which reacts with a plasma constituent to form active thromboplastin. It was postulated that the plasma factor is the precursor of thromboplastin and the name, thromboplastinogen, was proposed. A deficiency of either platelets or thromboplastinogen results in a lack of active thromboplastin, and therefore in poor consumption of prothrombin. The residual quantity of the latter in the serum may be utilized as a quantitative means for measuring the available thromboplastin in the blood.

While it is generally accepted that various tissue extracts contain active thromboplastin, little is known concerning the actual potency of such preparations. In the present investigation the prothrombin consumption test was employed to determine the available thromboplastin in the standardized rabbit brain extract which is used for the one-stage prothrombin time. The surprising finding was made that such a tissue extract which brings about prothrombin consumption in both hemophilic and normal deplateletized plasmas will, when heated to 60° C., lose its activity to cause prothrombin consumption in hemophilic plasma but remain highly active in normal deplateletized plasma.

EXPERIMENTAL

Blood was collected with a chilled silicone-coated syringe and needle, and transferred to silicone-coated test tubes immersed in an ice bath. To obtain a platelet-rich plasma, the

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blood was centrifuged at 800 to 1,000 r.p.m. for five minutes. Usually 70 per cent or more of the platelets remained in the plasma. The platelet-poor plasma was prepared by centrifuging blood at 8,000 r.p.m. for ten minutes in an Angle centrifuge. Such a plasma was found to contain about 3,000 platelets per cubic millimeter.

Acetone-dehydrated rabbit brain extract: (Thromboplastin reagent) 200 mg. of dried rabbit brain prepared according to the directions previously described[2] were mixed with 5 ml. of 0.85 per cent sodium chloride solution. The mixture was incubated at 50° C. for twenty minutes. A second mixture was incubated at 60° C. for twenty minutes.

Deprothrombinized rabbit plasma: Fresh oxalated rabbit plasma (9 volumes of blood mixed with 1 volume 0.1M sodium oxalate) was treated with tricalcium phosphate as follows: 5 ml. of a 0.012M tricalcium phosphate suspension were transferred to a test tube and centrifuged at 2,500 r.p.m. for three minutes. The supernatant water was completely removed, and 5 ml. of fresh oxalated rabbit plasma added to the packet adsorbent. After thorough mixing and allowing the tube to stand five minutes, the tricalcium phosphate was removed by centrifuging ten minutes at 2,500 r.p.m. The clear plasma was poured off and kept in an ice bath. It failed to clot when treated with thromboplastin reagent and calcium chloride.

Prothrombin determination by the adsorption and elution procedure: The method of Quick and Stefanini[3] was employed.

The prothrombin consumption test of plasma: To a clean dry test tube (13 by 100 mm.) containing four small glass beads, 2 ml. of native plasma were added. The tube was put in a water bath at 37° C. The clotting time was noted, and fifteen minutes later the clot was loosened from the sides of the test tube. After fifteen minutes further incubation, the clot was compressed and the prothrombin time of the serum determined as follows: to a mixture of 0.1 ml. thromboplastin reagent, 0.1 ml. 0.02M calcium chloride and 0.1 ml. deprothrombinized rabbit plasma, 0.1 ml. of the serum was added by blowing from a serological pipette. The formation of the clot was accurately timed. After ten minutes the determination was repeated. The prothrombic activity almost always becomes constant in less than twenty-five minutes after centrifugation. The serum remaining was analyzed for prothrombin by adsorption with tricalcium phosphate and elution with sodium citrate.[3]

In testing the thromboplastic activity of rabbit brain extract, 0.05 ml. of the latter was transferred to a test tube containing four small glass beads. To this 2 ml. of platelet-

TABLE I. EFFECT OF HEATING TISSUE EXTRACT AT 60° C. ON ITS THROMBOPLASTIC ACTIVITY IN NORMAL AND IN HEMOPHILIC PLATELET-POOR PLASMAS

	PROTHROMBIN CONSUMPTION TIMES OF SERUM IN SECONDS								
	NORMALS			HEMOPHILIACS			THROMBOCYTOPENIC PURPURA		
SOURCE OF SERUM	1	2	3	1	2	3	1	2	3
Platelet-poor plasma	8 (8)*	8 (8)	8 (8)	8 (8)	8 (8)	8 (8)	8	8	8
Platelet-rich plasma	16 (16)	18 (17)	25 (26)	8 (8)	10 (10)	11 (11)	11§	11§	10§
Platelet-poor plasma 2.00 c.c.									
Rabbit brain extract (50° C.†) 0.05 c.c.	19 (17)	66 (30)	48 (35)	32 (26)	28 (22)	12 (12)	37		
Platelet-poor plasma 2.00 c.c.									
Rabbit brain extract (60° C.‡) 0.05 c.c.	19 (17)	34 (28)	36 (33)	8 (8)	10 (9)	9 (8½)	29	31	44

*The figures in parentheses are the prothrombin times obtained by the adsorption-elution procedure.

†Incubated at 50° C. for 20 minutes.

‡Incubated at 60° C. for 20 minutes.

§Platelet counts: subject 1, 42,000; subject 2, 36,000; subject 3, 42,000.

poor plasma were added. Mixing was effected by inverting the test tube. After fifteen minutes of incubation at 37° C., the clot was loosened, and the procedure from then on was the same as outlined for the prothrombin consumption time of plasma.

The effect of adding rabbit brain extract (heated to 50° C. and to 60° C.) on the prothrombin consumption of normal and hemophilic plasma: From the results in Table I, it can be seen that rabbit brain extract heated to 50° C. when added either to normal, to hemophilic platelet-poor plasma, or to plasma from patients with thrombocytopenic purpura caused a marked consumption of prothrombin as shown by the prolonged prothrombin time of the serum. When heated to 60° C., the brain extract still retained its power to cause prothrombin consumption in normal and in thrombocytopenic plasmas, but became almost inactive in hemophilic plasma. The prothrombin consumption in platelet-poor plasma of normal subjects was extremely low, but in normal platelet-rich plasma it was relatively high.

TABLE II. PROTHROMBIN CONSUMPTION OF HEMOPHILIC BLOOD TO WHICH A FIXED AMOUNT OF RABBIT BRAIN EXTRACT HEATED TO 60° C. AND INCREASING AMOUNTS OF NORMAL PLASMA WERE ADDED

Hemophilic plasma (platelet-poor), c.c.	2.00	1.75	1.50	1.25	1.00	0.75	0.50	0.25	0
Normal plasma (platelet-poor), c.c.	—	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00
Rabbit brain extract heated to 60° C., c.c.	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Prothrombin consumption time direct, sec.	8	18½	28	41	43	45	50	48	48
Prothrombin consumption time of eluate, sec.	8½	18	28	35	36	36	36	36	35

TABLE III. QUANTITATIVE RELATIONSHIP BETWEEN THE PROTHROMBIN CONSUMPTION TIME OF PLATELET-POOR NORMAL PLASMA AND THE QUANTITY OF RABBIT BRAIN EXTRACT ADDED

Platelet-poor normal plasma, c.c.	2.0	2.000	2.00	2.00	2.00	2.00	2.00	2.0
Volume of rabbit brain extract, c.c.	0.0	0.005	0.01	0.02	0.03	0.04	0.05	0.1
Rabbit brain extract heated to 50° C.								
Prothrombin time direct, sec.	8	8	9	11½	23	35	42	64
Prothrombin time eluate, sec.	7½	8	9	14	26	35	43	45
Rabbit brain extract heated to 60° C.								
Prothrombin time direct, sec.	8	8	9	10	14½	20	23	27
Prothrombin time eluate, sec.	7½	8	9	11	16	24	29	38

Heated rabbit brain thromboplastin as a means to estimate the corrective action of normal plasma on hemophilic plasma: Rabbit brain when heated to 60° C. lost its potency to bring about consumption of prothrombin in hemophilic but not in normal plasma. On adding increasing amounts of normal plasma and a fixed quantity of heated rabbit brain extract to hemophilic plasma, the utilization of prothrombin increased, until it reached a value which was the same as that of the normal plasma in Table II. It should be observed that one part of normal in seven parts of hemophilic plasma produced a distinct increase in prothrombin consumption.

The thromboplastic potency of rabbit brain extract as measured by the prothrombin consumption test: The results recorded in Table III show that little prothrombin was consumed when platelet-poor plasma was clotted, and that addition of rabbit brain extract brought about a marked increase in utilization of prothrombin. The greater the amount of brain extract added, the more prothrombin was consumed. The extract heated to 60° C. when tested on normal platelet-poor plasma retained a high thromboplastic activity, but was somewhat less effective than the preparation incubated at 50° C.

DISCUSSION

The results obtained in the present study have both theoretical and practical significance. The findings suggest that thromboplastin should still be regarded as an activity rather than as a specific chemical compound. The term, thromboplastin, may therefore be best defined as the agent or group of agents which with calcium and labile factor are essential for the conversion of prothrombin to thrombin. Circulating blood contains no thromboplastin. It is formed only after platelets have undergone lysis and have liberated a factor which apparently reacts with a plasma constituent which we have designated, thromboplastinogen. For convenience and simplicity it has been postulated that the platelet factor and thromboplastinogen interact to form active thromboplastin. According to this concept, the first reaction in coagulation is:

Thromboplastinogen + platelet factor = thromboplastin

This reaction is hypothetical. It is employed primarily to express the fact that thrombolastic activity depends on a plasma constituent which is deficient in hemophilia, and also on a platelet factor. The sequence with which these agents react with labile factor and prothrombin remains undetermined. But it is an established fact that when much prothrombin remains in the serum (provided the blood is clotted under standardized conditions) a deficiency of either thromboplastinogen or of the platelet factor is usually responsible. The prothrombin consumption test becomes a simple means for measuring the over-all thromboplastic activity, but it fails to distinguish between the plasma and platelet factors.

It has generally been assumed that tissue extracts contain thromboplastin, which, in terms of the new concept, means both the plasma and the platelet factors. The results of the present study confirm this assumption, for if rabbit brain extract (incubated at 50° C.) is added to either normal platelet-poor plasma or to hemophilic plasma, prothrombin consumption occurs proportional to the amount of reagent added. This clearly shows that rabbit brain contains both factors, or the product of these two agents, namely, free thromboplastin.

A significant change occurs when tissue extract is heated to 60° C. or higher. While such an extract still causes normal prothrombin consumption in platelet-poor plasma from a normal subject or one with thrombocytopenic purpura, it fails to induce conversion of prothrombin to thrombin in hemophilic plasma. Heating therefore destroys the true or complete thromboplastic activity of tissue extract. This indicates that a preparation such as rabbit brain extract either contains both the primary factors that constitute thromboplastic activity, or free or active thromboplastin, if one assumes that the two agents interact to form a finite compound. On heating, thromboplastic activity is destroyed; but a factor which can react with the plasma factor, thromboplastinogen, remains fully active. Therefore heated rabbit brain is still highly active in normal plasma, but inactive when added to hemophilic plasma since the latter lacks thromboplastinogen.

The heat stable component of tissue extract behaves like the platelet factor which is also heat resistant. One can conclude that the platelet principle and the heat stable component of tissue extract are either identical or closely related physiologically. They are probably not proteins or enzymes as one of us[1] postulated. The results of the present study indicate that the heat stable component of tissue extract and thromboplastinogen interact stoechiometrically. Shinowara[4] has recently also concluded that the thromboplastic cell component and the plasma component react stoechiometrically.

Although the trustworthiness of the prothrombin consumption test is beginning to be recognized clinically, the reliability of the one-stage method to determine prothrombin in serum, nevertheless, has been questioned. The poor or slow conversion of prothrombin in hemophilia is, however, also demonstrable by the two-stage method. Brinkhous[5] already reported this is 1939. Soulier[6] and Merskey[7] who employed a modified type of two-stage method also confirmed the observation that much prothrombin remains in hemophilic serum. To meet the objections to the one-stage method, the prothrombin was determined by an alternate method, the adsorption and elution procedure.[3] It has been found that the removal of prothrombin from plasma or serum by adsorption to tricalcium phosphate and its subsequent elution with sodium citrate is sufficiently complete to permit employing the procedure for the quantitative determination of prothrombin. The agreement between the results obtained by the direct one-stage test and the adsorption-elution procedure are remarkably good for both normal and pathological bloods. This indicates that even if serum should contain accelerators or inhibitors, they have a negligible influence on the prothrombin time. It will be noted, however, that when tissue extract such as rabbit brain is added to plasma, a discrepancy may occur in the results obtained by the direct one-stage method and by the adsorption and elution procedure especially in the lower prothrombin range. In this type of experiment, the adsorption-elution test is the more reliable.

Irrespective of the theoretical implications of the present findings, the observation that rabbit brain extract heated to 60° C. loses its power to cause prothrombin conversion in hemophilic plasma, has proved to be of great value in the diagnosis of this disease. In addition to the patients studied in our laboratory, the senior author has applied the test in the examination of twelve hemophiliac patients in various countries of South America with uniformly good results. In thrombocytopenic purpura, the addition of rabbit brain extract heated to 60° C. brings about a high consumption of prothrombin, which follows logically since the thromboplastinogen is normal in this condition.

SUMMARY

Acetone dehydrated rabbit brain, which has a high thromboplastic activity as estimated by the one-stage prothrombin test, brings about consumption of prothrombin when added to platelet-poor normal or hemophilic plasma. When the extract is heated to 60° C., its activity to bring about utilization of prothrombin in hemophilic plasma is lost, but it remains active in platelet-poor

normal plasma. These findings suggest that a tissue extract such as that of brain has a heat labile factor which acts directly with prothrombin, and a heat stable agent which requires a plasma factor to produce active thromboplastin. In hemophilia the plasma factor is lacking. The ineffectiveness of heated rabbit brain extract to cause consumption of prothrombin in hemophilic plasma is important for the diagnosis of hemophilia.

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LABORATORY METHODS

THE DETERMINATION OF BIOLOGIC SODIUM

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THE determination of sodium in biological fluids has been the subject of many investigations and reports. The direct determination of sodium by use of uranyl triple acetate is the most common chemical method utilized. Many modifications of this method have been advanced.[1-5]

In our studies of base excretion on patients on low sodium diets we used the method suggested by Albanese and Lein,[1] as it appeared to be a simple and reliable one. However, when urinary sodium results using the Albanese method were compared to those found by use of a flame photometer (Barclay) discrepancies were noted. These errors and their correction are the subject of this report.

REAGENTS

Two reagents were used in this investigation, the reagent suggested by Albanese (hereafter referred to as the "original reagent") and a modification of this reagent (hereafter referred to as the "modified reagent"). The modified reagent is prepared from a stock reagent. This stock reagent is prepared by dissolving 100 Gm. of $UO_2(C_2H_3O_2)_2 \cdot 2H_2O$ in 500 ml. of boiling water containing 20 ml. of glacial acetic acid. In a separate container 300 Gm. of $Zn(C_2H_3O_2)_2 \cdot 2H_2O$ are dissolved in 500 ml. of boiling water containing 10 ml. of glacial acetic acid. The two boiling solutions are mixed and reheated just to boiling. This solution is cooled, filtered, and stored in a dark bottle out of direct sunlight. It is stable for months. The working reagent is prepared by diluting 5 volumes of stock reagent with 3 volumes of reagent methanol and 2 volumes of 95 per cent ethanol. It is filtered immediately before use.

RESULTS

Albanese reported that a plot of optical density vs. concentration of sodium resulted in a straight line. As this is in conflict with other reports,[2, 3] it was deemed necessary to investigate this calibration curve. Fig. 1 shows the calibration curve obtained in our investigation.

A large number of urine sodium determinations were carried out using the flame photometer and the Albanese method. A large positive error was noted in the chemical method in certain abnormal urines. Albanese has also noted this difference in urines of patients receiving ACTH or cortisone† (See

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*Part of M.S. thesis submitted by Merle R. Likins to Graduate School of the University of Louisville.

†Personal communication.

TABLE I. CHEMICAL ANALYSIS COMPARED TO FLAME ANALYSIS ON URINE

CHEMICAL	FLAME			APPROX. %
Na	Na	K	K/Na	ERROR
1.49	0.67	1.29	1.93	122
0.89	0.36	0.65	1.81	147
0.82	0.38	0.54	1.42	116
1.78	1.39	1.74	1.25	28
1.83	1.55	1.91	1.23	18
2.47	1.90	2.14	1.12	30
2.40	1.86	2.07	1.11	29
2.04	1.75	1.93	1.10	17
2.78	2.44	2.61	1.07	14
2.20	1.71	1.73	1.01	29
2.48	2.00	1.99	1.00	24
2.06	1.68	1.44	0.86	23

Chemical procedure according to Albanese.
Concentration expressed as grams per 24 hours.

Table I). This error seemed to be caused by the presence of abnormally high K/Na ratios. Accordingly, standard sodium solutions were contaminated with known amounts of potassium and the sodium determined by the original Albanese procedure. As the K/Na ratio approached unity, it was noted that the solutions became turbid due to the precipitation of the K uranyl salt (See Table II).

TABLE II. EFFECT OF K ON THE ALBANESE PROCEDURE FOR NA DETERMINATION

C_{Na}	C_K		AVERAGE
(MG. PER TUBE)		K/Na	O.D.*
0.40	0.0	0.0	0.329
0.40	0.1	0.25	0.332
0.40	0.2	0.50	0.331
0.40	0.3	0.75	0.344†
0.40	0.4	1.00	cloudy
0.80	0.0	0.0	0.565
0.80	0.2	0.25	0.564
0.80	0.6	0.75	0.568
0.80	0.8	1.00	cloudy

Optical Densities measured on a Coleman Universal spectrophotometer at 430 mμ. Precipitation was carried out at 4° C. for a period of one hour.
*Triplicate analyses. Precision of ± 1.5 per cent on clear solutions.
†One tube of three showed marked turbidity.

It was further noted that the original Albanese method gave dependable results on serum samples. Results of analyses by this method were compared to results obtained by flame analysis and are listed in Table III.

TABLE III. DETERMINATION OF SODIUM IN SERUM

CHEMICAL ANALYSIS	FLAME ANALYSIS
(MG. OF SODIUM PER 100 ML.)	
306.0	308.1
287.3	286.0
300.5	307.0
292.1	292.9
326.0	331.0
334.0	333.0
301.0	298.0
276.0	283.0

Chemical procedure followed was that recommended by Albanese.

Several modified reagents were investigated. However, the only satisfactory reagent proved to be the modified reagent described above. This reagent was tested for interference due to potassium and was found to be satisfactory. Several slight modifications in procedure were found necessary and will be discussed in a later portion of this paper. The decreased effect of potassium on the sodium determination is illustrated in Table IV. It should be noted in Table IV that the critical K/Na ratio using the modified reagent is greater than 25, whereas from Table II the K interference is noted when the K/Na ratio is approximately 0.75 using the Albanese procedure.

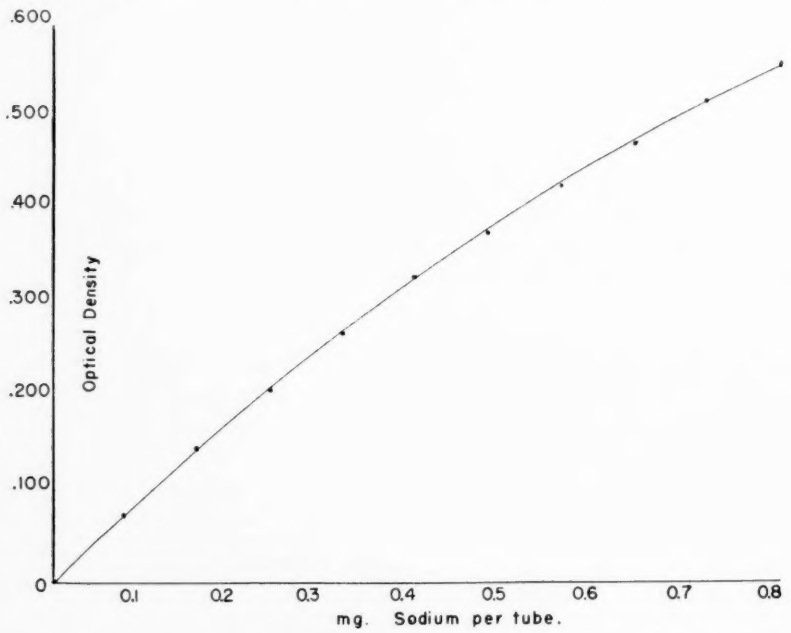

Fig. 1.—Calibration curve using Albanese procedure 430 mμ 19 by 105 mm. curvette.

TABLE IV. EFFECT OF K ON THE DETERMINATION OF NA USING THE MODIFIED REAGENT

C_{Na}	C_K	$\frac{C_K}{C_{Na}}$	AVERAGE O.D.*
0.4	0.0	0.0	0.339
0.4	0.2	0.5	0.343
0.4	0.4	1.0	0.342
0.4	0.6	1.5	0.342
0.4	4.0	10.0	0.342
0.4	8.0	20.0	0.345
0.4	10.0	25.0	0.339
0.8	0.0	0.0	0.600
0.8	0.4	0.5	0.596
0.8	0.8	1.0	0.605
0.8	1.2	1.5	0.594
0.8	8.0	10.0	0.596
0.8	16.0	20.0	0.597
0.8	20.0	25.0	0.602

The above data were obtained by analysis of 0.2 ml. of solution by addition of 2.0 ml. of modified reagent. The precipitation was carried out at room temperature for a period of two hours.

*Triplicate analyses. Precision of ± 1.5 per cent.

Table V contains data comparing values obtained on pathological urines by the modified method and flame analysis.

The procedure used for the modified reagent varies from that of Albanese in three respects only. (a) For each volume of unknown 10 volumes of reagent are used for precipitation. (b) The time needed for precipitation is two hours instead of one hour. (c) The precipitation is carried out at room temperature rather than at 4° C.

TABLE V. DETERMINATION OF Na BY THE MODIFIED REAGENT AS COMPARED TO FLAME ANALYSIS ON ABNORMAL HUMAN URINE

GM. OF Na/24 HRS. BY CHEMICAL METHOD	GM. OF Na/24 HRS. BY FLAME METHOD
1.05	1.05
1.28	1.32
0.57	0.55
0.30	0.31
2.90	2.93
0.78	0.79
3.71	3.65
2.15	2.24
0.91	0.96
1.68	1.69

The procedure followed in obtaining the above data was that described for the modified reagent.

DISCUSSION OF RESULTS

The discrepancy between the calibration curve obtained by Albanese and the one obtained in this laboratory can be partially accounted for by the difference in instruments used. (Klett-Summerson photoelectric colorimeter with an S-42 filter vs. Coleman Model 14 spectrophotometer.) Since at this point the Albanese calibration curve is being discussed it should be pointed out that the formula reported by Albanese to replace the calibration curve is not in accordance with mathematical facts. In order for such a formula of proportionality to be used the calibration curve must be a straight line of such nature that it can be projected through the origin. The curve reported by Albanese does not meet this qualification; hence, to use the proportional formula in his method, multiple standard solutions must be run with the unknown so that the difference in optical densities between the unknown and standard is small.

The interference of potassium was first noted when certain urines showed a cloudiness after being centrifuged to free the solution of uranyl phosphate. At first this was thought to be due to excess phosphates, but examination supported the fact, reported by Albanese, that phosphates offer no interference. It soon became evident that these abnormal urines were those having a high K/Na ratio. Experiments were undertaken to establish the critical ratio where potassium interfered with the determination. This ratio was found to be extremely variable using the Albanese method. However, it was determined that if the K/Na ratio was smaller than 0.5, no interference occurred. In average normal urine this ratio would rarely be exceeded. When the ratio was between 0.5 and 1.0, duplicate or triplicate analyses could not be repeated consistently due to the presence of K uranyl acetate. Some tubes would be cloudy, some had normal optical densities, and some resulted in clear solutions with

optical densities of higher values than theoretical. K/Na ratios larger than 1.0 always resulted in cloudy solutions. If these cloudy tubes were allowed to stand overnight and centrifuged, a clear solution was obtained, but error due to high optical densities was observed.

The uranyl crystals obtained with standard sodium solutions using the Albanese reagent are smaller in size and darker yellow than those obtained with potassium. In fact, the laboratory personnel working daily with this determination could observe the uranyl precipitates after washing with alcohol and tell if mixed Na and K uranyl crystals were present.

It was apparent that in certain pathologic conditions or in cases of individuals on a restricted salt diet the Albanese method would not yield reliable urine sodium values. Shorter precipitation periods and higher precipitation temperatures were investigated as a means of correcting this error in the Albanese method; both results were only partially successful. The critical K/Na ratio was improved, but it was still not high enough for unrestricted use.

It was found that the modified reagent required ten volumes of reagent to each volume of unknown solution in order to give dependable results, and that for all precipitation two hours were required. Precipitation carried out at 4° C. with the modified reagent caused a smaller critical K/Na ratio than that at room temperature. The critical K/Na ratio of 25 obtained with the modified reagent and technique is large enough so that difficulty due to high K/Na ratios should never be encountered in even the most pathologic urine. The data in Table V illustrates the accuracy of the modified method compared to flame analysis.

CONCLUSION

The method of sodium analysis offered by Albanese is satisfactory when a calibration curve is used for calculation, but determinations are limited to serum or normal urine analysis. For pathological urines with a high K/Na ratio it is necessary to use a modified reagent.

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VISUAL DELINEATION OF THYROID GLANDS IN VIVO

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THIS report describes the visual delineation of thyroid glands of patients to whom I^{131} had been administered. The visualization of the area occupied by the gland is accomplished by means of an automatic scanning device and a visual recorder[1] which registers gamma ray activity.

It is suggested that this type of picture or visualization of a radioactive area be called a "scintigram" and that specifically in this case they be called "thyroid scintigrams."

Allen, Libby, and Cassen[2] have recently described an in vivo method for outlining the area occupied by the thyroid gland. A directional scintillation counter[3] is used to determine the count rate for a series of coordinate points over and around the area occupied by the gland. By drawing a line through the series of isocount points representing the margin of the lobes of the thyroid, an outline of the gland is obtained. This method, while affording excellent delineation of the area occupied by the thyroid gland, proved tedious both for the patient and the nurse or technician recording the observations because of the time required to complete a series of some thirty to sixty individual recordings of the count rate. This procedure has been greatly improved and simplified by the development of an automatic scanning device and a visual recorder.[1] The time required to complete an outline of the gland was one to two hours, whereas a "scintigram" can be obtained mechanically in from fifteen to thirty minutes.

The detailed construction of the automatic scanner and the visual recorder will appear in a separate report.[1] Briefly, the automatic scanner consists of a carriage support for the directional scintillation counter.[2] The counter is moved alternately back and forth, with a 1/8 inch vertical displacement for each sweep or scan over the area occupied by the thyroid gland. This movement is represented by the solid lines appearing in the "scintigram." (See Figs. 3 to 6.) The movements of the directional counter are controlled by an arrangement of electric motors, gears, screws, and micro switches. Mechanically, there are a number of efficient designs that will provide this type of motion.

The visual recorder is mounted over a 7 inch by 8 inch metal drawing board and is an integral part of the automatic scanner (Figs. 1 and 2). The recorder consists of a writing pen actuated by a small 110 volt electromagnet. It is similar to the chronograph pen attachment used on Esterline-Angus graphic instruments for recording the time of occurrence and duration of some event which is related to the record being made by the

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instrument.* The 110-volt pulse that activates the electromagnet and pen of the visual recorder is provided through the output leads of a laboratory scaling circuit of the type employed for counting radioactive samples. In our laboratory, both a decimal and a binary scaling circuit have been used. It is advantageous to provide a selection of scales, since no two thyroid glands will have an identical amount of radioactivity. Our experience to date would indicate that the best "scintigram" of the active area is obtained when the count is at least five to ten times greater than the background count observed outside the radioactive gland. The density of the "scintigram" is determined by the radioactivity in the gland, the rate at which the directional scintillation counter is moved across the active area and the scale (2, 4, 8, 16), etc.) selected. The rate of scan and the selection of the scale that provide the best visualization are determined by a single preliminary scan across the radioactive thyroid area. The scaler which activates the pipping pen should be set in such a way that individual pen strokes are recorded, and that smearing of the individual strokes is avoided. The scanning distance is adjusted so that a nonactive area outside each lobe is covered during each movement of the directional scintillation counter over the patient's neck.

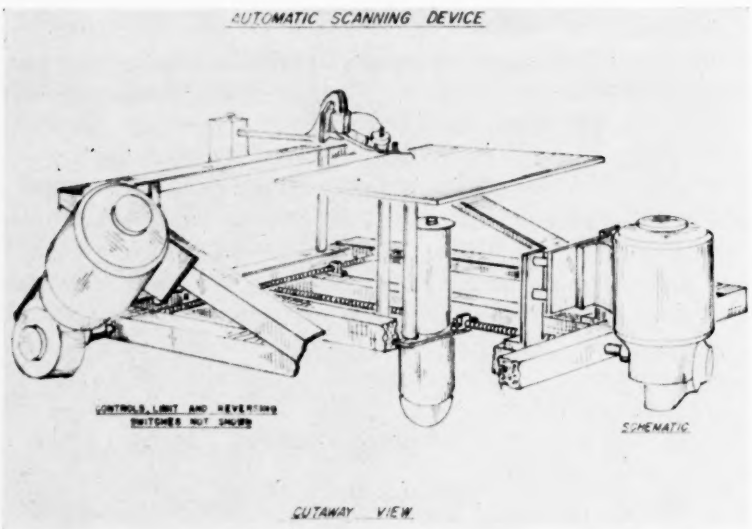

Fig. 1.—Diagrammatic view of essential components of automatic scanner.

The principle involved in obtaining the "scintigram" is relatively simple. As the directional scintillation counter scans over a nonactive area of the neck, the writing pen records each pulse fed to it by the scaling circuit by drawing a 1/8 inch line (Figs. 3 to 6). The spacing between each line represents the time for the scaler to accumulate a predetermined number of counts. As the directional scintillation counter scans over the radioactive area of the neck, the count rate increases, and thus the spacing between the lines drawn by the visual recorder decreases if a constant rate of scan is maintained. If a complete survey of the patient's neck is made, one obtains "scintigrams" similar to those shown in Figs. 3 to 6.

*Esterline-Angus, List of Supplies, Accessories and Parts for Model AW Graphic Instruments, Cat. No. 8510.

The automatic scanner and visual recorder have been extremely useful in a number of instances which will be discussed as follows:

A. It permits a complete visualization of functioning thyroid tissue whether in the thyroid or in other areas of the body (the instrument can probably be adapted to other isotopes). The "scintigram" of the gland has been invaluable in our efforts to arrive at the correct estimation of therapeutic doses of I^{131} in the treatment of hyperthyroidism. In most institutions the weight of the gland is estimated by palpation. We have been attempting to arrive at the correct weight by calculating the area of the gland, multiplying this area (centimeter square) by the maximum length in centimeters and a constant factor which, in principle, represents the ratio of the average thickness of the gland to the length of the gland, multiplied by the specific gravity of thyroid tissue. This factor has been derived from the study of several surgical and postmortem thyroid specimens.[4] The area of the gland and the maximum length are obtained directly from the "scintigram"; the area is measured with a planimeter.

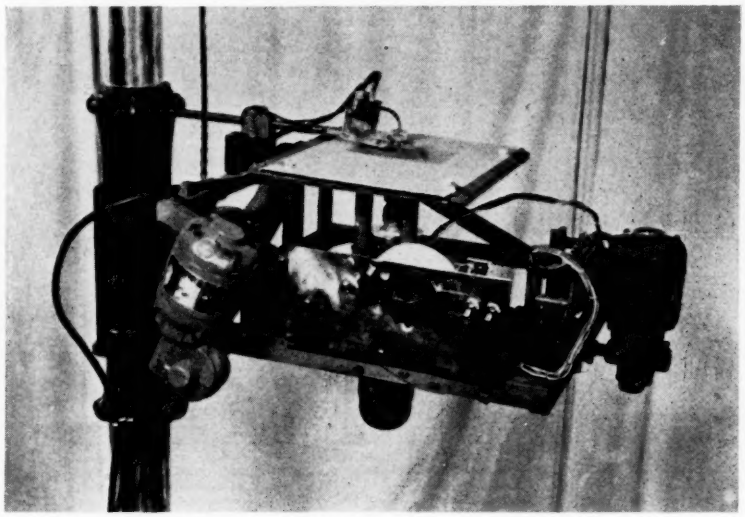

Fig. 2.—View of experimental automatic scanner.

B. It is possible to differentiate abnormally functioning portions of the thyroid gland. We have observed several instances where portions of the thyroid gland took up more I^{131} than the rest of the gland although no nodes could be palpated clinically or were even suspected. If nodes are palpated the instrument can determine whether these nodes consist of functioning thyroid tissue, at least as far as their iodine concentrating ability is concerned. The fact that unsuspected "nodular" areas can be found seems to us to be of importance in cancer prevention.

C. A series of "scintigrams" of several patients who had received therapeutic doses of I^{131} for hyperthyroidism were done. The doses ranged from 2 to 12 millicuries, and tracings were obtained biweekly. Swelling and tender-

ness of the thyroid gland have been mentioned in the literature following administration of therapeutic doses of I^{131}. In none of our patients has this been observed clinically and the "scintigrams" show considerable decrease in functioning gland areas in the second week following therapy.

This is illustrated by the case of C. B., a 27-year-old man with typical Graves' disease. (See Figs. 3 to 6.)

D. We have followed six patients with cancer of the thyroid who had had radical neck dissection for recurrence of thyroid tissue. In one patient we

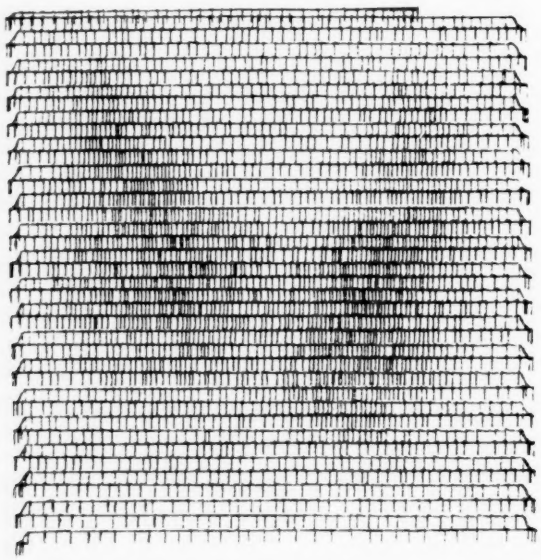

Fig. 3.—"Scintigram" of thyroid gland obtained twenty-four hours after administration of a tracer dose of 200 microcuries of carrier-free I^{131}. Each pen stroke represents 16 pulses from the scintillation tube.

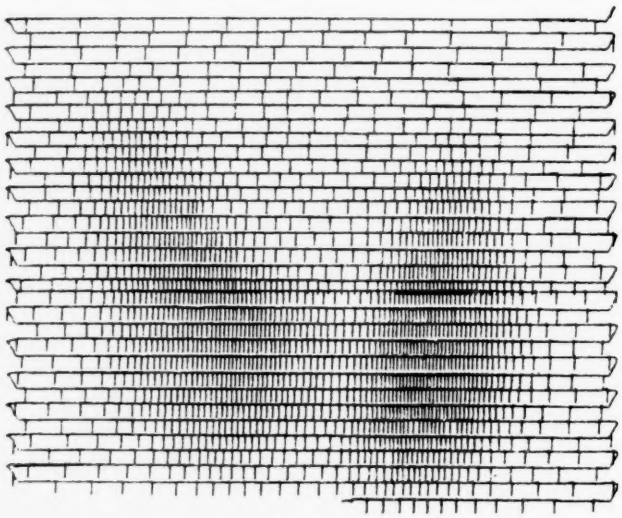

Fig. 4.—"Scintigram" of thyroid gland six days after therapeutic dose of 8.75 millicuries of carrier-free I^{131}.

were able to determine the presence of a small node before this node could be felt by palpation. The node was removed and found to be a metastatic lesion.

E. We are adapting the instrument for the concurrent determination of accumulation of I^{131} in the thyroid gland (uptakes) as well as outlining the thyroid gland so that a complete study can be done in forty-eight hours. In

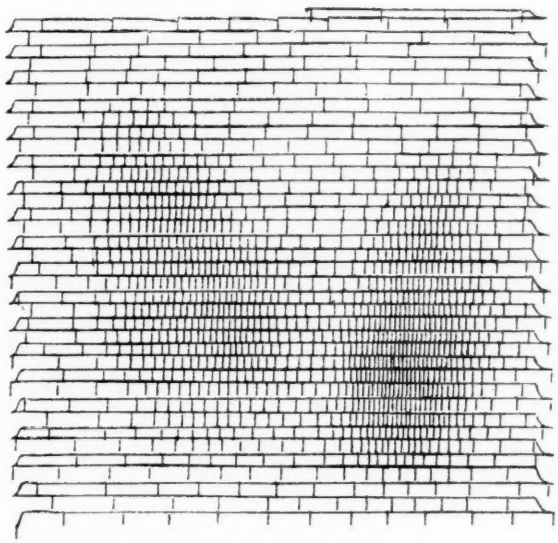

Fig. 5.—"Scintigram" of thyroid gland twelve days after therapy; in this figure and in Fig. 6, each stroke represents 128 pulses from the scintillation tube. The amounts of I^{131} remaining in the gland at this time are in the millicurie range compared to the tracer dose in Fig. 3. Note slight shrinkage of gland.

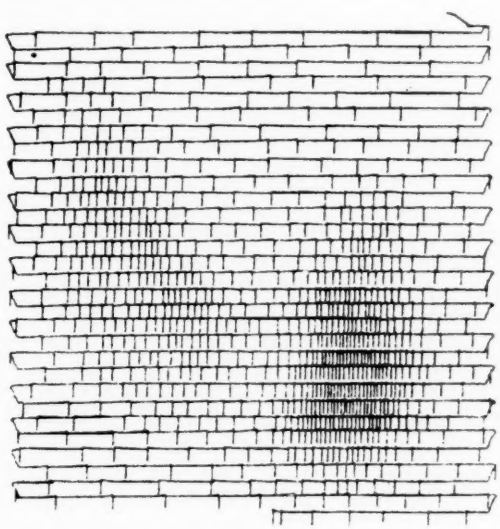

Fig. 6.—"Scintigram" of thyroid gland twenty days after therapy; note shrinkage of functioning area of thyroid gland. The high concentration of I^{131} in the lower portion of the left lobe suggests a reutilization or translocation of I^{131} from some other source. Studies to explain this phenomenon are in progress.

the past, the uptake study and outlining procedure had to be done consecutively because the dose used for the uptake study was too small for the directional scintillation counter on the automatic scanner and the scanning dose too large for the wide angle scintillation tube.[2] This instrument can be used to visualize radioactive areas other than the thyroid gland, and can be adapted to other gamma emitting isotopes.

SUMMARY

The clinical usefulness of an automatic scanner and visual recorder for delineating thyroid glands containing I^{131} has been demonstrated. It is suggested that this type of visualization of a radioactive area be called a "scintigram."

The instrument has proved helpful in the estimation of gland size and weight. It permits visual follow-up of I^{131} therapy in patients with hyperthyroidism.

Nodes which accumulate I^{131} can be differentiated from those which do not. Clinically unsuspected areas which concentrate more I^{131} have been demonstrated.

The instrument is valuable in the follow-up of thyroid cancer patients. It enables the surgeon to be certain of complete removal of functioning thyroid tissue.

William D. Harris, B. S., and Mrs. J. Perini, R.N., gave technical assistance.

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A SIMPLIFIED METHOD FOR DETERMINING THE EXCRETION RATE OF UROPEPSIN

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THE growing interest in the relationship between adrenal cortical function and various diseases in man has made it desirable to discover relatively simple methods for estimating the output of Compound E and F type hormones. Spiro, Reifenstein, and Gray[1] have shown that the administration of ACTH to a normal individual causes an increase in the secretion of gastric pepsinogen. As demonstrated by Mirsky and associates,[2, 3] this substance is released into the circulation and appears in the urine as uropepsin, an identical or closely related enzyme. In Addison's disease uropepsin excretion is low, is not increased by ACTH, but returns to normal when cortisone is given.[1] These and related findings are significant in that they suggest that the excretion rate of uropepsin is under hormonal control mediated via the adrenal cortex. In order to interpret the results of uropepsin assays in terms of adrenal activity, it is of course necessary that the target organ, the gastric mucosa, be responsive to stimulation. As many investigators have reported[1, 3, 4, 5] uropepsin is consistently absent from the urines of patients with pernicious anemia or following total gastrectomy.

MATERIALS AND METHOD

The method to be described is a modification of that originated by Sylvest[6] for determining the renninogen content of urine. In its present form, uropepsin assays may be made rapidly with a minimum of equipment and technical experience. In this clinic the procedure has yielded consistently reliable results.

Reagents.—(a) pH 4.90 acetate buffer (NaOH 4.2 Gm., glacial acetic acid 9.2 ml., distilled water to make 100 ml.). Adjust pH accurately using pH meter.
(b) 2N HCl.
(c) 0.2 per cent aqueous methyl orange.
(d) Milk-buffer mixture: equal parts of fresh homogenized milk and acetate buffer.

Collection of Timed Urine Samples.—The patient is instructed to empty his bladder, discard this specimen, and record the time. The next voiding is saved, the total volume

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measured, and the time again noted. If the collection is to extend beyond six hours, toluene should be added. Specimens thus preserved may be stored in the refrigerator for two weeks without losing activity.

Activation of Urinary Pepsinogen.—Pepsinogen is converted to pepsin by the addition of 0.05 ml. methyl orange and 0.10 ml. 2N HCl to 2.0 ml. aliquots of the timed urine samples. The resulting mixture should then be acid to methyl orange, i.e., pH 3 or less. Occasionally more HCl will be required, in which case the final result is corrected for the dilution error. Next, the acidified urine is incubated in a water bath at 37° C. for one hour. Activation of pepsinogen takes place rapidly in the first fifteen minutes, and is usually complete in thirty minutes. At one hour pepsin activity has reached a maximum which is unchanged by further incubation.

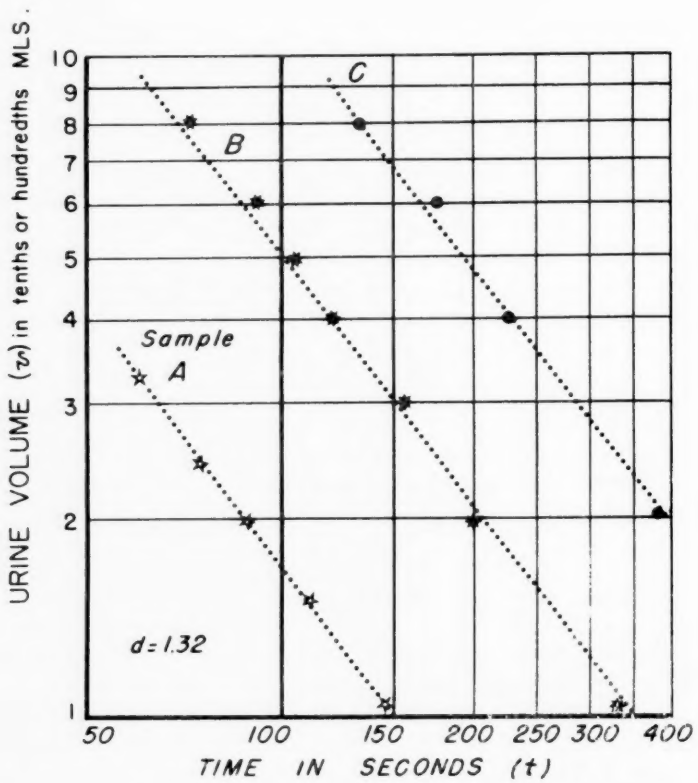

Fig. 1.—Relationship between enzyme reaction time and uropepsin content of urine samples.

Measurement of Uropepsin Concentration.—All reagents used including distilled water are brought to 37° C. To a test tube add 0.10 ml. activated urine, 0.90 ml. distilled water, and 1.0 ml. acetate buffer. Mix thoroughly. The reaction is then started by the addition of 0.5 ml. milk-buffer, the tube is shaken quickly, a stop watch started, and the tube placed in the 37° C. water bath. The end point of the reaction, aggregation of casein particles, is very sharp and can be read with an accuracy of less than five seconds. The reading is made in the thin film on the inner walls of the tube above the surface of the mixture, and for this reason as the end point is reached the tube should be shaken slightly every ten to fifteen seconds to facilitate detection of the protein precipitation. In this way the amount of acidified

urine which produces an end point in eighty to four hundred seconds is determined. If the values obtained using 0.1 ml. of the sample fall on either side of this range, the test is repeated with smaller or larger amounts, adjusting the volume of distilled water added so that the tube contains exactly 1.0 ml. before the addition of buffer. The amount of urine used may vary between 0.01 and 1.0 ml., but in the majority of specimens, 0.1 ml. will be found to give the desired reading.

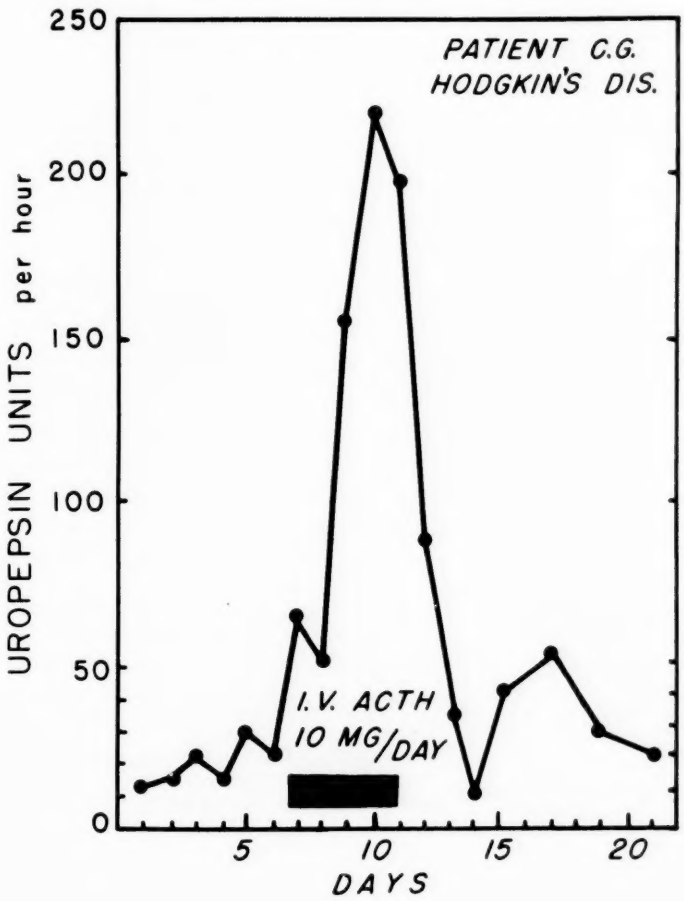

Fig. 2.—Stimulation of uropepsin excretion by adrenocorticotrophic hormone.

RESULTS

Results are expressed in terms of units of uropepsin excreted per hour, one unit being defined for reasons of convenience and to facilitate comparison with data obtained by other methods as the number of milliliters of activated urine × 10 required to give the end point in 100 seconds. This is equivalent to 0.26 micrograms of crystallized pepsin (Armour). Since the volume of urine used in the determination and the time in seconds required to reach the end point are

logarithmically related (Fig. 1) and when so plotted yield a straight line with a slope, y/x, of 1.32, it follows that the units of uropepsin excreted per hour may be expressed

$$\text{Units/hr.} = \frac{1}{10} \times \frac{V}{vh} \left(\frac{100}{t}\right) 1.32$$

where v=volume in ml. used in the test, t=seconds required to reach the end point, V=total volume of the timed specimen, and h=hours during which V was secreted. Inasmuch as the equation is not suitable for operational use without log tables or log-log slide rule, the graphical solution is very convenient. On log-log graph paper draw lines of standard slope back to the 100 second abscissa from any vt point determined by experiment. The value of v at this intercept is the number of milliliters of urine which will give the end point in 100 seconds. Using this value, v', the calculation is simplified as follows:

$$\text{Units/hr} = \frac{V}{v' \times h \times 10}$$

By this method, the morning excretion rate of uropepsin in healthy individuals is found to be 15 to 40 units per hour, with little variation in the same subject from day to day. Afternoon and night values are lower. The administration of ACTH may result in excretion rates in excess of 200 units per hour (Fig. 2).

SUMMARY

A convenient method for determining the rate of uropepsin excretion is described. The procedure is suitable for detecting increases in production of this enzyme which are known to accompany adrenal cortical stimulation.

The authors wish to thank A. G. Van Alstyne, Gilfillan Bros., Inc., Los Angeles, for assistance in developing the equation on which the calculation of results is based.

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NOTE ON THE STABILITY OF CREATININE IN NEUTRAL AND ALKALINE URINE

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Philadelphia, Pa.

PRESERVATION of bile[1, 2] and porphyrin[3] pigments for determinations in urine collected over twenty-four hour periods is effectively accomplished by the presence of sodium carbonate (5 Gm.) and petroleum ether. Precise interpretation of the analytical results requires that such collections actually represent the total urine secreted by the patient within the twenty-four hour period, yet the obstacles encountered when this is attempted are well known. Measurement of creatinine excretion as a means of evaluating uniformity of urine collections offers helpful information. However, some doubt exists as to the stability of creatinine, especially in alkaline solution, over a period of one or more days. Ellinger and Matsuoka[4] claimed that 10 per cent of the creatinine in a solution containing 0.75 per cent sodium carbonate was destroyed at 37° C. within two weeks. Brazda, Espenan, and Beard[5] stated that creatinine was transformed to creatine at pH 6.7 to 7.6 at 37° C. Horvath and Dill[6] found a similar conversion to creatine occurred in normal urine, containing thymol as preservative, at room temperature and in the cold. They advocated analysis within twenty-four hours of the collection period.

Table I. Stability of Creatinine in Urine Preserved with Petroleum Ether and Sodium Carbonate. Findings in Urine of Eight Male and Seven Female Laboratory Workers

Additions to urine	Days stored	Creatinine mg./100 ml. average concentration	Precision*
None	Freshly voided	167	1.3
Petroleum ether	2	169	1.7
	3	169	1.5
	7	170	1.5
Plus 0.33 % sodium carbonate	2	164	1.8
	3	170	1.1
	7	164	2.3
Plus 1 % sodium carbonate	2	164	1.5
	3	171	1.5
	7	165	1.5

*The precision of the determinations was calculated using the expression

$$\sigma = \sqrt{\frac{(X_1 - X_2)^2}{N}}$$

where n is the total number of determinations (two times the number of pairs) and $X_1 - X_2$ represents the difference between duplicates.

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From the Pepper Laboratory of Clinical Medicine and Harrison Department of Surgical Research, University of Pennsylvania.

The work reported in this paper was done in connection with studies sponsored by the Army Epidemiology Board under the commissions on Liver Diseases and under contract with the Department of the Army.

We have tested the stability of creatinine in urine in the presence of sodium carbonate and petroleum ether, and find that no change occurred under conditions ordinarily used in making such collections. Single voidings of urine from fifteen laboratory workers were analyzed promptly after collection. Aliquots were preserved at 5 to 8° C. with petroleum ether. To aliquot (a), no further additions were made. To aliquot (b), sodium carbonate sufficient to make the concentration 0.33 per cent was added, and in aliquot (c), the sodium carbonate concentration was increased to one per cent. The pH of the fresh urine varied between 5 and 7. The pH of the (b) series varied from 6 to 8, and that of the (c) series from 7 to 10. Determinations of creatinine were made by the method of Bonsness and Taussky,[7] two, four, and seven days later.

The results are summarized in Table I. It may be seen that differences between fresh and stored samples were slight and without statistical significance.

SUMMARY

Urine containing sodium carbonate in amounts used for preservation of bile and porphyrin pigments showed no change in creatinine concentration when stored seven days at 5 to 8° C. in the presence of petroleum ether.

Eleven of fifteen urine specimens stored as described retained all creatinine after four weeks, and only one lost more than 7 per cent.

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TRANSPARENT HOLDER FOR SMALL ANIMALS

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DURING a recent series of experiments in this laboratory it proved necessary to inject large numbers of rats subcutaneously several times a day for prolonged periods of time. In order to facilitate this procedure, a transparent plastic holder was devised, shown in Fig. 1, that permitted one operator to inject the rats at a rate of approximately three animals per minute.

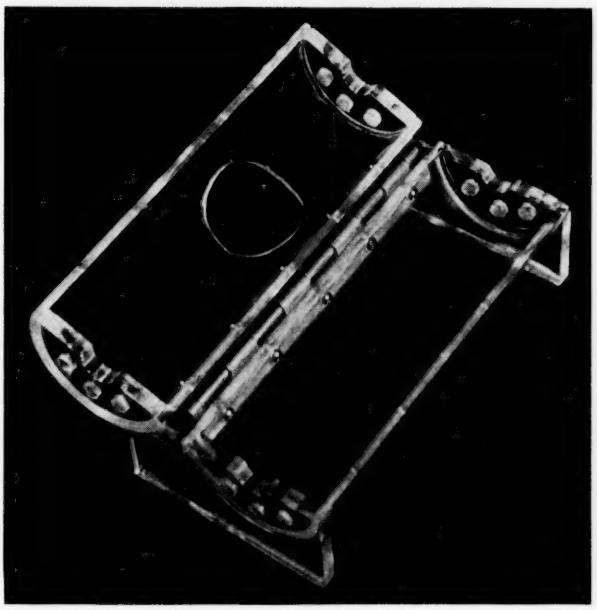

Fig. 1.

Construction.—The body of the holder is made from a transparent plastic (Lucite) tube. The diameter and length of the tube depend on the size of the animals to be injected. In practice it was found that a tube 2¼ inches in diameter and 6½ inches long would accommodate rats weighing 150 to 220 grams. Discs are cut from sheet plastic to fit in the ends of the tube. The discs are drilled with a half-inch hole in the center, through which the tail emerges, and a series of smaller holes to serve as air holes. The ends are cemented into the tube, using acrylic solvent, and the cylinder is split lengthwise. A base

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is fitted on the lower shell and cemented in place. A hole is cut in the upper shell for injecting the animals subcutaneously. The location and size of the hole depend on the type and location of injections to be made. For most injec-

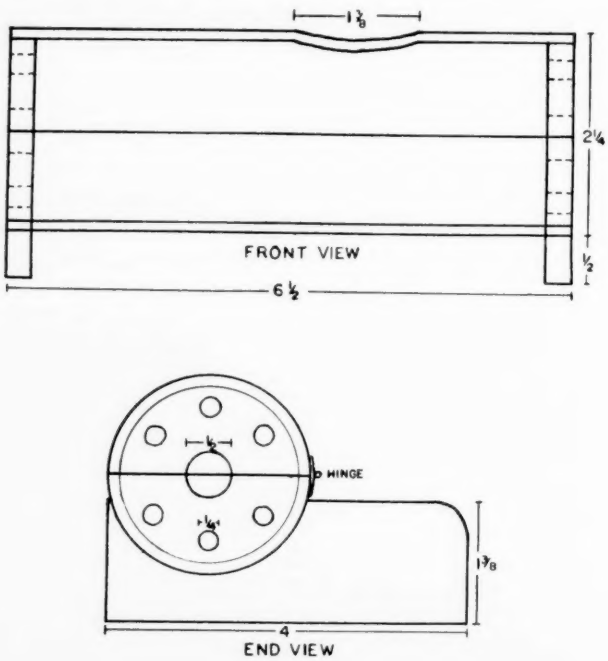

Fig. 2.

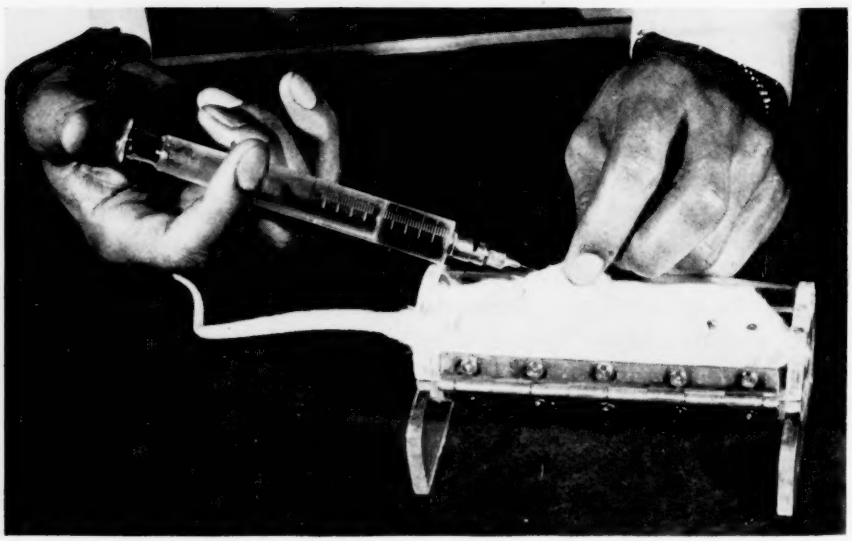

Fig. 3.

tions a hole $1\frac{3}{8}$ inches in diameter located just posteriorly to the center of the holder is satisfactory. A length of brass piano hinge is bolted along the back of the holder to form a bivalve structure as shown in Fig. 2.

Use.—Subcutaneous injections are readily made in rats using this holder. The animal is simply placed in the holder and the top is closed. A fold of skin is pulled up through the hole in the top of the holder and the injection is made into and under this fold, as seen in Fig. 3.

Intravenous injections are easily made in the tail of the rat which extends through the hole in the end of the holder. Dilatation of the vein is accomplished by picking the holder up and briefly immersing the animal's tail in warm water.

Continuous intravenous drip administration of fluids is also facilitated by the holder. In this case the animal is placed in the holder and the top is taped shut. The tail is then fatsened to a splint and the tail vein is dilated. The needle is placed in the vein and taped in position. Such injections have been done repeatedly in rats with no apparent ill effects.

Intraperitoneal injections can also be done utilizing the same holder. The rat is placed in the holder, and then the holder is inverted. This causes the rat to right himself and thus the abdominal wall presents itself in the injection window. A fold of skin is grasped to immobilize the animal and the holder is righted. The injection is then easily accomplished through the abdominal wall.

The disadvantages of anesthetizing the animal can be avoided in many procedures by using the holder. Blood pressures in the rat are easily obtained by immobilizing the rat in the holder; this gives a truer value of the blood pressure than is obtained when the animal is anesthetized or tied down. Also an animal can be bled repeatedly in a single day where repeated anesthesia might prove harmful.

DISCUSSION AND SUMMARY

This small holder has several advantages over those that have heretofore been used. Its greatest advantage is that it permits one operator to inject the animals rapidly. Second, since the tube is slightly larger than the rat, the animal is not pinched in any way and, while he is almost completely immobilized, there is no danger whatsoever of damaging the animal. The transparency of the holder facilitates injecting since the animal is completely visible at all times. Since the holder is made of easily worked plastic it can be constructed by anyone with a few hand tools. The holder is easily kept clean, for all seams are cemented and the plastic may simply be wiped off. The holder may be readily modified to accommodate either mice or guinea pigs.

A LUER SLIP WITH A ROTATING LOCK

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THE recording of pressure-pulse contours has come into widespread use. Lead tubing with Luer slips is frequently used for making connections in such work. However, a male Luer-Lok slip can be used only at one end of the lead tubing. The connection at the other end, especially if the tubing is short, has to be a plain male Luer slip, and this end has a habit of working loose at the most inopportune times. To overcome this difficulty, a Luer slip with a

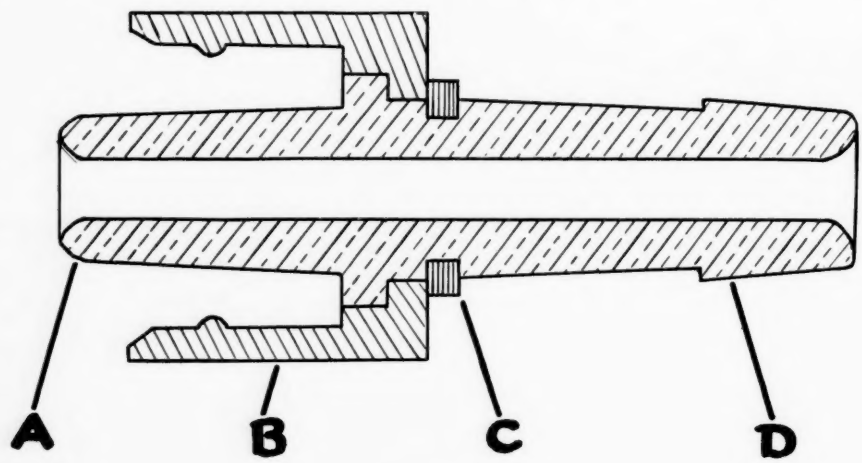

Fig. 1.

rotating lock was devised. It differs from the regular Luer-Lok in that the locking portion rotates and can be tightened without rotating the Luer slip and lead tubing. This rotating Luer-Lok slip was machined by combining the locking portion of a male Luer slip with a regular male Luer. In Fig. 1, *A-D* is the regular male Luer, *A* is the male Luer slip, and *D* is the end soldered to the lead tubing; *B* is a rotating locking portion, and *C* is a snugly-fitting washer to hold *B* in place. This Luer slip with a rotating lock has worked very satisfactorily.

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